Grams:UNICOLENG **Tel:040-27098213**

DEPARTMENT OF ELECTRONICS AND COMMUNICATION ENGINEERING
UNIVESITY COLLEGE OF ENGINEERING (AUTONOMOUS)
OSMANIA UNIVERSITY, HYDERABAD-500 007

A Report on

Computational Tools for Segmentation, Enhancement, Edge Detection and De-noising of Confocal Brain Images of Fruit Fly

Submitted by

Dr. P. Ananth Raj, Emeritus Fellow
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ABSTRACT

One of the important problems in image processing is segmenting an image into different non-overlapping regions using one of the two properties of image intensity values: discontinuity and similarity. In the first category, image is portioned into different regions based on abrupt changes in intensity, such as edges. In the second category, image is split into different regions that are similar, based on some property, thresholding, region growing and region splitting are some examples of this category. Among, all these segmentation approaches, thresholding techniques are more simple, efficient and more suitable for bi-level image segmentation task. Hence, we have developed different thresholding methods that use both one dimensional and two dimensional histograms and different types of entropies for thresholding an image into a bi-level image.

Further, we have also employed, C-Means and Fuzzy C-Means algorithms for segmenting a confocal Fruit Fly brain images into two-region images. From the above study, we can conclude that 2D histogram, Gray level co-occurrence matrix approaches and Fuzzy clustering approaches performed better than other approaches. Further, it is observed that, one of the Fruit Fly brain images captured at the surface level, do not produced a good contrast image. Hence, we have proposed a Gaussian Hermite polynomial based image enhancement method that performed better than other moment-based methods.

Quite frequently, images are corrupted by Gaussian noise; different filters such as mean, median, Non-local mean and bilateral filters are proposed to correct it. In this work, we propose a novel filter which use both non-local mean filter and orthogonal moments for filtering noisy confocal Fruit Fly brain images. It is found that, the proposed filter produced good results for those images that are corrupted with a small amount of noise. Another important problem in image segmentation is detection of edges; therefore, we have employed Zernike moments for detection of edges in confocal images and compared it with Sobel and Robert edge detectors.

In general, our study focused on segmenting a confocal image using two different approaches namely, detection of edges using Zernike moments and segmenting using c-means, fuzzy c-means and thresholding approaches, which use similarity criteria for image segmentation

CHAPTER 1

Introduction

The main objective of image segmentation is to partition an image into different non-overlapping regions. Until today, many research papers and review articles have been published in National and International journals [1,2,3,4,5,6,7,23a,23b,23c] . In general, available methods are broadly grouped into Histogram based techniques, Edge based techniques, Region based techniques, Markov Random field-based techniques, Graph theory-based Techniques, clustering techniques, neural network based and Fuzzy set theory- based methods. Among all these techniques, histogram-based methods are widely used because they these are simple and provides good results for bi-level segmentation task. In this report, we explain different histogram based image segmentation techniques that use spatial and non-spatial information based histogram and different types of entropies for segmentation task. Further, we discuss how they perform for confocal microscope images. Even though, these methods are applicable to multilevel segmentation, but our study was focused on two level image segmentation problem only.

The main objective of edge-based techniques is to calculate edge points using standard edge detectors like Sobel, Canny, Gradient, Laplacian of Gaussian operators (LOG). Next these edge points are linked to form an object boundary. Boundary based approaches also called an Active Contour model also known as Snakes was introduced by KASS et al [8]. It is a deformable curve whose shape is modified iteratively to obtain the object contour by minimizing a cost function that consists of both external and internal energies. Internal energy serves as smoothness constrain and external energy guides the active contour towards the image features that minimize the total energy of the contour. Some of the drawbacks of the above methods are a) wrong selection of initial contour that needs more number of iterations. b) Final contour is estimated using an iterative technique. Hence, global solution is not guaranteed. Therefore, to correct these drawbacks many modifications were suggested in the literature [9,10]. Fuzzy energy minimization of active contour using multi resolution approach were proposed by Stelious Krinidis and Vassidis, Chatzis [10] to reduce the computational time as well as to obtain a better solution. Another approach for estimation of object contour using level sets was proposed by Osher and Sethian [11]. The main advantage of using level sets is, intrinsic representation and topological changes can be handled.

Region based approaches attempt to partition the given image directly into regions based on the pixels, color or texture that satisfy certain predefined homogeneity criteria. Some of the well- known region based techniques are region growing, region splitting and merging [12] and morphological watersheds and JSEG algorithms proposed by Deng and Manjunath [13,14] . Region growing is a procedure that groups pixels or sub-regions into larger regions based on a predefined criterion for growth, the basic approach is to start with the set of "seed" points and from these seed points, regions are grown by appending to each seed those neighboring pixels that have predefined properties similar to the seed point. C-means algorithm works on the above-mentioned category. Splitting and Merging is another region-based technique that subdivides an image initially into a set of arbitrary disjoint regions and merge and/or splits the regions in an attempt to satisfy the basic segmentation rules. Splitting techniques tends to produce boundaries consisting of long horizontal and vertical segments. Recently, a region merging technique was proposed by Felipe Calderero and Ferran Marques [15] based on information theory.

Markov random field -based techniques assume that the true image is a realization of a Markov or Gibbs random field with a distribution that captures the spatial context of the scene. Given the prior distribution of the true image and the observed noisy one, the segmentation problem is formulated as an optimization problem. Optimal solution is obtained by using any one of the global optimization techniques like simulated annealing or genetic algorithm. Many research articles were published on this topic **[16]**.Watershed segmentation is one of the traditional region- based approaches [12]. This method is based on visualizing an image in three dimensions: two spatial coordinates versus intensity. This technique segments an image into several catchment basins, which are the regions of an image (interpreted as a height field or landscape) where rain would flow into the same, an efficient way to compute such regions is to start flooding the landscape at all of the local minima and to label ridges wherever differently evolving components meet. This algorithm is usually applied to a smoothed version of gradient magnitude image because images rarely have dark regions separated by lighter ridges. This method is sensitive to morphological structuring element. Another disadvantage of this method is no control of the smoothness of the segmentation results. Another drawback is that it does not permit incorporation of a priory information in the segmentation problem. These drawbacks are addressed in by Hieutat et al [17] in their research paper.

Recently, a number of Graph based approaches were developed to solve image segmentation problem. The main advantage of these methods is the absence of discrete error and availability of efficient tools for graph theory. In this approach image segmentation is achieved by cutting the graph into different regions. One of the first graph based approaches with a global cost function was proposed by Wu et.al.[18a,18b , 19], after that, many efficient methods were reported in the literature using different cost functions that consists of Ratio cut [20] Normalized cut[21], Minimal cut [24], Mean-shift [22] etc. In this approach a weighted undirected graph is constructed from a given image where each pixel becomes vertex of the graph, it is a fully connected graph, each weight is computed by a function that measures the similarity between the vertices, Low level features such as pixels, colors and textures are used for estimating the similarity. All these methods provide mixed results for different classes of image data.

In order to extend these methods for pixels having vagueness while classifying pixels either to Class 1 or Class 2 in a two class problem, a large number of image segmentation methods using Fuzzy sets were proposed in the literature [25,26,28,30,33a, 33b]. These methods are classified into five categories namely, Fuzzy clustering methods, Rule based methods, Fuzzy geometrical methods, Information theoretical methods and Type II thresholding methods [27,29,31a,31b,32]. Fuzzy clustering techniques cluster all image pixels into different segments based on some distance measure. The most commonly used fuzzy clustering techniques are c means and fuzzy c means [34] , collaborative fuzzy clustering methods. Fuzzy rule based methods [35**]** use Fuzzy if then rules for threshold determination. Fuzzy geometrical methods minimize or maximize fuzzy geometrical measures such as compactness for image segmentation [36]. Information theoretical methods minimize or maximize the fuzzy entropy and its extensions such as Tsallis entropy [37,38,39,40,41,44] Renyi's entropy [42,43], Havrds and Charvad [37] entropies. Type II Fuzzy thresholding methods[27,29,31a, 31b] interpret image information as Type II fuzzy sets and use information theoretical measures such as fuzzy entropy or its extensions or index of ultra-fuzziness to identify global threshold value. Some of the recent works reported on this topic address the use of Fuzzy-2 partition entropy[31], interval type-2 fuzzy entropy[32], multilevel image segmentation[45] and Type II fuzzy sets[31a,31b,32] for hand written Chinese character recognition problems.

Another class of work reported on image segmentation was based on C-means, Fuzzy C means and possibilistic [46,34] algorithms. In these methods an objective function is

constructed using the sum of differences between the data points and cluster centers. This function is minimized to obtain a segmented image. This formulation does not provide good results for noisy images due to the absence of filter term in the objective function. Hence, most of the research papers included a filter term to solve the above problem. An excellent survey paper on clustering algorithms was reported by Rui Xu and D.Wunsch II [47] covering Graph theory based, neural network based, Fuzzy and non fuzzy clustering algorithms in detail. Another survey paper on clustering algorithms was reported by B. Vinodhini [48]. Another recent paper addressed the problem of noisy images was published by Feng Zhao et. al in [49] that covers image segmentation using Fuzzy C-Means and their extensions . In order to provide good results for noisy images, Bilateral and patch-based filter terms were included in the objective function by different authors [50].Most of the image segmentation algorithms presented above provides mixed results. Hence to improve the results, two or more techniques are integrated. Recently, for accurate classification of Hyperspectral images Mean shift segmentation technique and histogram- based techniques were combined [51]. Another paper on multilevel image segmentation using Fuzzy entropy and Graph Cut optimization was reported in their research paper [52]. Benjamin Schmid [122] in his PhD thesis developed ***Computational tools for the segmentation and registration of confocal brain images of Drosophila melanogaster***. This work has several differences from our work like, we used thresholding methods and clustering methods for segmentation, whereas Benjamin Schmid used active contour models for segmentation..Further, we have used orthogonal moments for edge detection and noise filtering of confocal images..

Histogram based thresholding methods are preferred over other methods, because they are simple, efficient and more suitable for bi-level image segmentation task. One dimensional histogram-based method does not use the local information of neighboring information. Hence, they do not provide satisfactory results. Therefore in order to improve the results, 2D histogram [53,54] and Gray Level co-occurrence matrix-based approaches-based approaches[55,56] were suggested. After this work, many 2D histogram based approaches were proposed using, image and local variance (GLLV) [57], image and its gradient magnitude (GLGM) [58] and image and number of neighboring pixels at each image pixel. Another, 2D histogram (GLSC) based approach was developed [59] using Gray-level Co occurrence matrix. Recently, Surima Borjigin and P.K.Sahoo [60] extended the 2D spatial domain thresholding, technique for multilevel color images using the particle Swam optimization and Tsallis-Havrda-Charvat entropy. Before the above work was published, Soham Sarkar et.al [40] published their work on multi-level image thresholding for monochrome images using differential evolution method, 2D histogram and cross entropy. Some more methods based on 2D histogram and different types of entropies are also published for monochrome images [62].Recently,3D Otsu's based thresholding techniques were proposed for multilevel image segmentation task[63]. In these methods a 3D histogram is constructed using the given image and its mean and median filtered images to obtain better results than 2D histogram methods. Further, this method will provide better noise immunity power than 1D and 2D histogram methods. Recently, another 3D Otsu's method [64] was proposed using the spatial context energy curve and mean and median filtered images for

color images. Few years back, a 1D median based thresholding method was proposed by Jing-Hao Xue [65], for monochrome images. This method is more robust to the presence of skew and heavy tailed image histogram because, it uses the mean absolute deviation from median for all data points in each class. All these methods worked well for different types of images. This observation made us to use them for confocal images.

1.1 Problem Description

A microscope is an imaging device that provides sufficient magnification and resolution of the test object. In a wide field microscope one obtains a signal/image with more background, because it collects light from all other planes. Hence, the contrast of the signal/image will be poor, whereas in a confocal microscope this problem was reduced using a pin hole mechanism. Therefore, we obtain a good quality image of the test object with less background. Further, it also provides a good localization / co localization of proteins present in the biological samples. It is also possible, to obtain 3D visualization of the test samples. In a confocal microscope the axial or Z resolution is limited to 500 nm. We can get a plane of 500 nm thickness using confocal microscope. The background present along with the signal/image within this 500 nm thickness can be reduced using image processing techniques. Confocal microscope can also be used to visualize a structure with 250nm lateral resolution at best conditions of the fluorescently labeled biological samples.

In order to study how different image segmentation techniques, work for confocal images of Fruit Fly brain image data, we procured. Data samples from CCMB, Hyderabad. In this work, we have considered image enhancement, Non–local mean filter developed using orthogonal moments, Histogram based spatial, non- spatial thresholding techniques, Zernike moments based edge detection and clustering techniques like c-means and Fuzzy c-means methods for our study purpose.

1.2. Organization of the Report

This report is organized into seven chapters. Second chapter, presents non-spatial thresholding methods, whereas third chapter, discusses spatial domain methods used in confocal image segmentation. Next chapter, presents moment based contrast enhancement method. Fifth chapter presents moment based image de-noising and edge detection methods. Sixth chapter discusses clustering methods. Last chapter presents the summary and conclusion about the work.

CHAPTER 2

Histogram Based Thresholding Techniques-I

These methods are grouped into either, spatial or non-spatial thresholding methods based on the use of spatial information or not. In this chapter, we present non-spatial thresholding methods that do not consider neighboring pixels, for segmenting the confocal images. Further, we also present simulation results on confocal images of a Fruit fly image.

2.1 Non-spatial thresholding methods

These methods do not consider the spatial relationship between the image pixels. Hence,in this chapter a brief description and simulation results are presented. Let G denote a set of all gray values $\{0, 1, 2, \ldots . L-1\}$ of the image *f*. Suppose that the image is known to contain k regions or objects say $R_1, R_2, R_3 \ldots . R_k$ Let P_i be the prior probability of region R_i where $i = 1,2,3 \ldots . k$ respectively. Let $\mu_i and\, \sigma_i^2$ be the mean and variance of region R_i.If $\sigma_1 = \sigma_2 = \sigma_3 = \ldots . . \sigma_k = \sigma$ then the single threshold value for two regions $(k = 2)$ [12] is

$$T = \frac{\mu_1 + \mu_2}{2} + \frac{\sigma^2}{\mu_1 - \mu_2} \log\left(\frac{p_1}{p_2}\right) \quad (1)$$

If the prior probabilities p_1 and p_2 are equal then the optimal threshold is the average of μ_1, μ_2

2.1.1 Ridler and Calvard algorithm [66] : It is an iterative method, consists of following steps:

1] Select the initial threshold (t_{init}) as the average gray-level of the image f

2] Segment the image into two regions (R_1, R_2) using the above threshold value

$$g(x,y) = \begin{cases} 1, & f(x,y) \geq t_{init} \\ 0, & other\ wise \end{cases}$$

3] Calculate the mean gray level of regions$R_1\ and\ R_2$, denote them as $\mu_1 and\, \mu_2$ respectively.

4] Calculate the new threshold value using

$$t_{new} = \frac{1}{2}(\mu_1 + \mu_2)$$

5] Repeat the steps 2,3 and 4 until the convergence is reached. When the algorithm stops, required threshold value is the average of the two mean values.

2.1.2 Kittler and Illingworth method [67]: This method assumes that an image is characterized by a mixture distribution with the population of object and background classes are normally distributed. The probability density function of the mixture distribution is estimated based on the histogram of the image. The main steps of the algorithm are

1] Choose an arbitrary initial threshold *t* and segment the image into two regions R1 and R2 respectively.

2] Compute $\mu_1(t),\ \mu_2(t),\ \sigma_1(t),\ \sigma_2(t),\ P_1(t),\ and\, P_2(t)$ i.e., class mean, class variance and class probability.

3] Compute the updated threshold by solving the following equation

$$g^2\left[\frac{1}{\sigma_1^2(t)}-\frac{1}{\sigma_2^2(T)}\right]-2g\left[\frac{\mu_1(T)}{\sigma_1^2(T)}-\frac{\mu_2(T)}{\sigma_2^2(T)}\right]+\frac{\mu_1^2(T)}{\sigma_1^2(T)}-\frac{\mu_2^2(T)}{\sigma_1^2(T)}+2\left[\log\sigma_1(T)-\log\sigma_2(T)\right]-2\left[\log P_1(T)-\log P_2(T)\right]=0$$

4] If the new threshold value equals the old one then terminates the algorithm else go to Step2.

If the class probabilities and class variance are equal optimal threshold value is equal to the expression given in Eq.1.

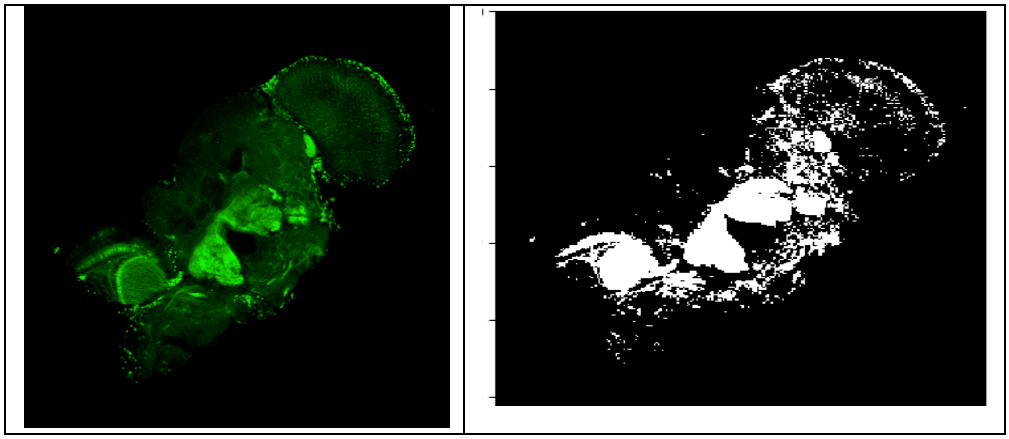

Figure 1. Bi_level thresholding using Ridler and Calvard algorithms Th=34

2.2. Lloyd's method: Lloyd suggested a modification of the iterative threshold selection method of Ridler and Calvard. This method is same as the above method. Except the step 4 is changed to

$$T=\frac{\mu_1+\mu_2}{2}+\frac{\sigma^2}{\mu_1+\mu_2}\log\left(\frac{n_1}{n_2}\right)\text{ here }\quad\sigma_1^2=\sigma_2^2=\sigma^2$$

n_1 and n_2 are the number of pixels in regions R_1 and R_2 respectively, Lloyd found that this modified iterative thresholding method converges to a satisfactory threshold value more often than the method of Calvard [66], However this method does not provide good results for noisy images.

2.3 OTSU's method [68,69]: This method determines the optimal threshold using discriminant analysis by maximizing the inter-class variation or equivalently minimizing intra-class variance[13]. A threshold *'t'* partitions the image into two regions R_1 and R_2 . Let the pixels in an image be represented in L gray levels. The total number of pixels at level L_i is denoted by N_i and the total number of pixels by $N=N_1+N_2+\ldots\ldots N_L$ gray level. In the case of bi-level thresholding of an image, pixels are divided into two regions. Let R_1 be the first class with gray levels [0, 1, 2 …t] and R_2 be the second class with gray-levels [$t+1$, t+2, L − 1] after thresholding. The probability of region R_1 and region R_2 is given by

$P_1(t)=\sum_{x=0}^{t}h(x)$,$P_2(t)=\sum_{x=t+1}^{L-1}h(x)$=1.0-$P_1(t)$where$h(x)=\frac{n_i}{N}$, x∈ $(0,1,2\ldots.(L-1))$

The histogram of the image is denoted by $h(i)$ can be computed by counting the frequencies of the intensities and dividing by the total number of pixels in the image.A general formula of the Otsu's method in the case of two regions is

$$\sigma_B^2(t) = arg \min_{0 \le t \le L-1} \left\{ p_1(t)\sigma_1^2(t) - p_2(t)\sigma_2^2(t) \right\}$$

Where $p_1(t)$ and $p_2(t)$ are the probabilities of pixels in regions $R_1(t)$ and $R_2(t)$ determined by threshold value t, $\sigma_1(t), \sigma_2(t)$, μ_1 *and* μ_2 are the standard deviation and mean values of region 1 and 2 respectively. Mathematical expressions for computing them are given by

$$\sigma_1^2(t) = \sum_{x=0}^{t} \frac{h(x)}{P_1(x)} [x - \mu_1(x)]^2 \,, \quad \mu_1 = \sum_{x=0}^{t} \left[x \left(\frac{h(x)}{P_1(x)} \right) \right]$$

$$\sigma_2^2(t) = \sum_{x=t+1}^{L-1} \frac{h(x)}{P_2(x)} [x - \mu_2(x)]^2, \quad \mu_2 = \sum_{x=t+1}^{L-1} \left[x \left(\frac{h(x)}{P_2(x)} \right) \right]$$

Optimal threshold is the case of two regions R_1 and R_2 is

$$Topt = \arg\max [\sigma_B^2(t)]$$

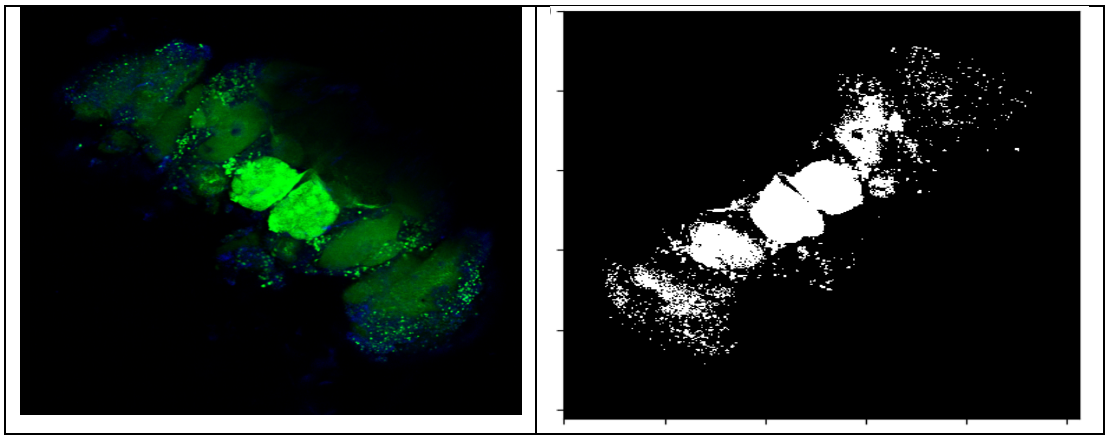

Figure 2 Bi-level segmentation of Fruit Fly image, Th=44

This method can be extended to more than two levels. In this work, we have also used Otsu's method for three level segmentation of confocal images. For three classes or three regions say, the R_1, R_2 *and* R_3, separated by two thresholds t_1*and* t_2, the between class-class variance is given by

$$\sigma_B^2(t_1, t_2) = P_1P_3(\mu_1 - \mu_3)^2 + P_1P_2(\mu_1 - \mu_2)^2 + P_2P_3(\mu_2 - \mu_3)^2$$

$$P_1 = \sum_{x=0}^{t_1} h(x), \; P_2 = \sum_{x=t_1+1}^{t_2} h(x) \; P_3 = \sum_{x=t_2+1}^{L-1} h(x) \; P_1 + P_2 + P_3 = 1$$

$$\mu_1 = \frac{1}{P_1} \sum_{x=0}^{t_1} xh(x), \; \mu_2 = \frac{1}{P_2} \sum_{x=t_1+1}^{t_2} xh(x) \; \mu_3 = \frac{1}{P_3} \sum_{x=t_2+1}^{L-1} xh(x)$$

Optimal thresholds for three classes are found by

$$\sigma_B^2(t_1^*, t_2^*) = \max_{0 \le t_1, t_2 \le L-1} \sigma_B^2(t_1, t_2)$$

The procedure starts by selecting the first threshold value t_1, next threshold value t_2 is incremented through all its values greater than t_2 and less than (L-1), then increment t_1 to its

next value and t_2 is incremented again through all its values greater than t_1, this procedure is repeated until $t_1 = (L-5)$, pick up (t_1, t_2) that gives the maximum value [12].

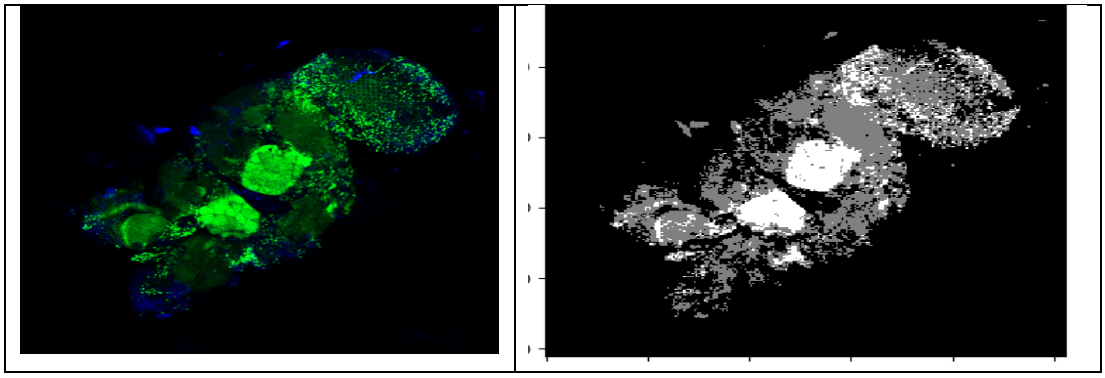

Figure 3.Three level segmentation of Fruit Fly image Th1-21, Th2=78

2.4. Iterative Tri-Class Image Segmentation: Otsu's method does not perform well if an image is dominated by the presence of one region pixels. In order to correct this limitation Hongmin Cai et. al. [70] proposed a recursive tri-class image segmentation. This method divides the image histogram into three regions namely, foregroundR_1, background $R_2$and To Be Determined (TBD) R_{12}regions using Otsu's threshold and means of the foreground and background regions. To Be Determined region is further divided into three regions. This process of dividing TBD regions continues until a single threshold values is obtained. Main steps of this algorithm are

1] Use Otsu's method to find a threshold value 't' at iteration one. This operation divides the image into two regionsR_1 and R_2.

2] compute the mean of R_1 and R_2, denote them as μ_1 and μ_2.

3] classify image pixels into three regions by applying a]R1 if f(x,y) $\leq \mu_1$ b] R_2if f(x,y) $\geq \mu_2$c] $R_{12}\mu_1$ <if f(x,y) $< \mu_2$

4] Stop the process until the difference between two consecutive threshold values is less than a preset threshold. Final threshold value is equal to the threshold value at this iteration.

2.5 Variable Thresholding Method**:** Performance of a threshold algorithm mentioned above depends on noise as well as image with non-uniform illumination. The tri-class method proposed by Hongmin Cai et. al.[70] demonstrated that their proposed approach provides good results for an image having non-uniform illumination. It is mentioned in the chapter 10 of the book [71] that there exists two methods namely, image partitioning and local image properties method, for threshold value estimation from images having noise and non-uniform illumination. In the image partition method, the given image is sub-divided into non-overlapping rectangles. These rectangles are chosen as small as possible such that the illumination of each region is approximately uniform. Otsu's method is applied to find the threshold value for each region. In the second method at every image position local mean, global mean and standard deviation is computed using a small neighborhood.

Let $\sigma_{x,y}$ and $\mu_{x,y}$ denote the standard deviation and mean value of a set of pixels in a neighborhood $\Omega_{x,y}$ centered at co-ordinates (x, y) in the image. The variable threshold at each point is

$$T_{x,y} = a\sigma_{x,y} + b\mu_{x,y}$$

Where a,b are non-negative constants or

$$T_{x,y} = a\sigma_{x,y} + b\mu_G$$

Where $\mu_G$the global image mean value, segmented image is is computed at each pixel

$$g(x,y) = \begin{cases} 1 & if\ f(x,y) > T_{x,y} \\ 0 & if\ f(x,y) \le T_{x,y} \end{cases}$$

This equation is evaluated for all pixel locations in the image to obtain a two level thresholded image

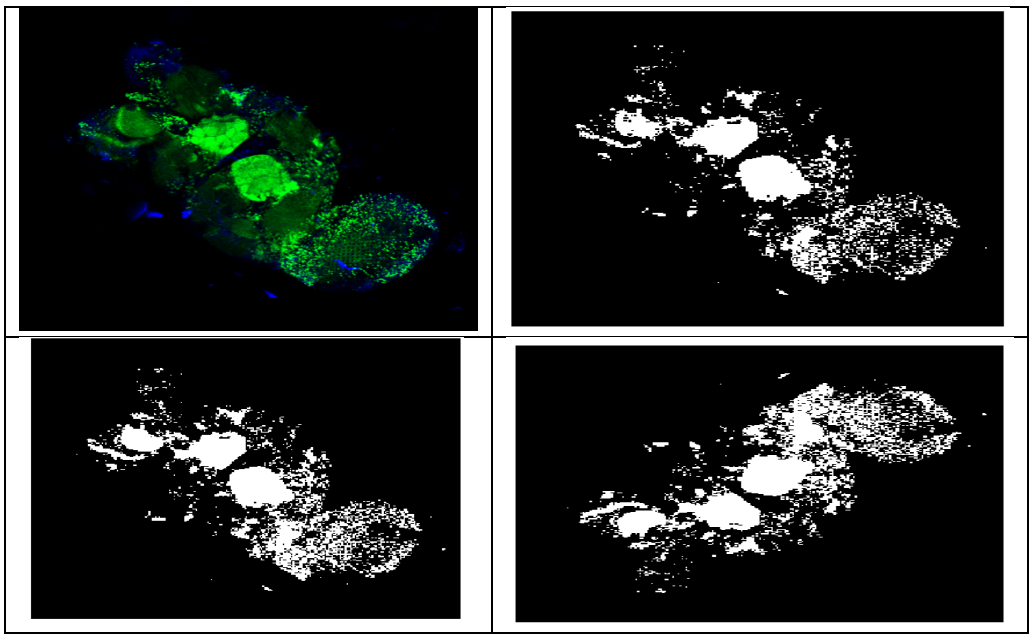

Figure 4. Bilevel segmentation using tri-class thresholding method a) Original Image (TL) b) bi-level image (TR) Th1=49 c) bi-level image (BL) Th2=39 d) Bi-level image (BR) Th3=35

2.6 Fisher linear Discriminant method**.** This method is the benchmark for linear discrimination between two classes in multidimensional space. This approach may not give minimum classification error unless the two classes are Gaussian with equal covariance matrix. Therefore Tristrom Cooke [72] proposed two fast and simple techniques for improving the classification performance of this method. Calculation of Linear Discriminant is simple and fast, because it requires only first and second distribution of two classes. Liyuan Li et al [73] proposed criteria based on two-dimensional histogram of the image. Recently, Gamil Abdel Azim and Z. A. Abo Eleneen [74] proposed a simple algorithm for image segmentation using the combination of within class variance and intensity contrast between the object and background. Recently, same authors reported another segmentation algorithm based on Fishers information measure [75] and applied it for medical images. The new error function E(t) that finds the maximum ratio of between class scatter to within class scatter is

$$E(t) = \sigma_w^{-2}(t) * (\mu_2(t) - \mu_1(t))^2$$

$$t^* = \max_{0 \le t \le (L-1)} E(t)$$

$$\sigma_w^2(t) = P_1(t)\sigma_1^2(t) + P_2(t)\sigma_2^2(t)$$

$$P_1(t) = \sum_{m=0}^{t} h(m),\ P_2(t) = \sum_{m=t+1}^{L-1} h(m)$$

$$\sigma_1^2(t) = \sum_{m=0}^{t} \left[\frac{h(m)}{P_1(t)}(m - \mu_1(t))^2\right],\ \sigma_2^2(t) = \sum_{m=t+1}^{L-1} \left[\frac{h(m)}{P_2(t)}(m - \mu_2(t))^2\right]$$

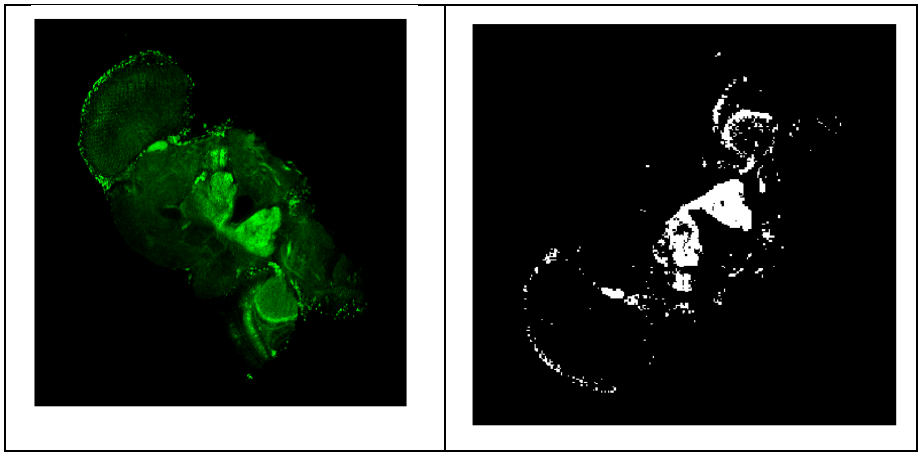

Figure 5 Image thresholding using Fisher thresholding method a) Original image b) Thresholded image Th=65

2.7 Simulation Results: In order to test all the methods described above, we have used the fruit fly brain images obtained using confocal microscope. These images are obtained from Center for Cellular and Microbiology, Hyderabad. It is known that we get horizontal slices of the test sample which are used for 3D shape reconstruction of the test sample. In this work we have selected few samples randomly for bi-level segmentation task. Threshold values obtained for various images are shown in the Figures 1 to 5. All the algorithms used in this work are developed using open CV and python software loaded on MacBook Air Laptop , Intel core I5 processor, 256 GB RAM. All the above methods segmented Central portion of the image very well. Further, processing is required to extract only the central portion of the Fruit Fly brain image. In figure 4 , TL, TR, BL and BR denotes top left, top right, bottom left and bottom right figures

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CHAPTER 3

Histogram Based Thresholding Techniques- II

Spatial thresholding techniques are another group of methods which use spatial information, belong to histogram-based thresholding technique's group. These methods use either 1D or 2D or 3D image histogram for finding the threshold point. Neighborhood valley emphasis method [76] and Median filter-based method [65] use 1D histogram, other methods, like 2D entropy methods use either 2D histogram matrix [39] or 2D gray-level co occurrence matrix [55]. Three dimensional, methods [63,64,77,78] use 3D histograms constructed using three images like given image, mean filtered image and median filtered image. The main objective of combining three images is to reduce noise. Next, we describe some of the above methods and apply them for confocal image binary segmentation task.

3.1 Neighborhood Valley Emphasis Method:

Valley emphasis method selects a threshold value with a small probability of occurrence that maximize the between class variance. This method does not provide good results if the histogram is unimodal or close to unimodal. In order to correct this error , Jiu-Lun Fan and Bo Lei [76] proposed a neighborhood valley emphasis method that uses neighborhood information at the threshold point as well as around the threshold point. The objective function for of the neighborhood valley-emphasis method for bi-level image segmentation is

$E(t) = (1 - \tilde{h}\,(t))(P_1(t)\mu_1(t) + P_1(t)\mu_2(t))$

The optimal threshold value is chosen by maximizing the above function. In the above expression $\tilde{h}(t)$ is the probability of occurrence of a pixel at gray value t, $\mu_1(t)$, $\mu_2(t)$ are the mean values of class 1 and class 2 respectively. $P_1(t)$, $P_2(t)$ are the probabilities of class 1 and class 2, defined in the last section. It is easy to extend, this formula for multilevel thresholding problem [76]. Figure 6. Show the result obtained with this method.

3.2 Median Based Thresholding Technique [65] : In order to make the above method more robust to the presence of skew and heavy tailed distributions of each class, Jing Hao Xue and D. Michael Titterington [61] proposed two methods using Valley Emphasis and Kittler and Illingworth's methods. Objective function for first method is

$$E(t) = P_1(t)MAD_1(t) + P_2(t)MAD_2(t).$$

Where $MAD_k(t)$is the mean absolute deviation from the median for class k and it is given by

$$MAD_1(t) = \sum_{x=0}^{t} \frac{h(x)}{p_1(t)} \left\{ x - m_1(k) \right\}, MAD_2(t) = \sum_{x=t+1}^{L-1} \frac{h(x)}{p_2(t)} \left\{ x - m_2(k) \right\}$$

where $m_1(t) = med[x_i : i \in R_1(t)]$, $m_2(t) = med[x_i : i \in R_2(t)]$

are the sample median for class1, $R_1(t)$ and class 2, $R_2(t)$respectively, Optimal threshold in the case of Kittler and Illingworth method is

$$E(t) = \left[P_1(t) \log \frac{MAD_1(t)}{P_1(t)} + P_2(t) \log \frac{MAD_2(t)}{P_2(t)} \right]$$

It is easy to extend these two methods for multilevel thresholding. Optimal threshold value is selected by minimizing the above two expressions.

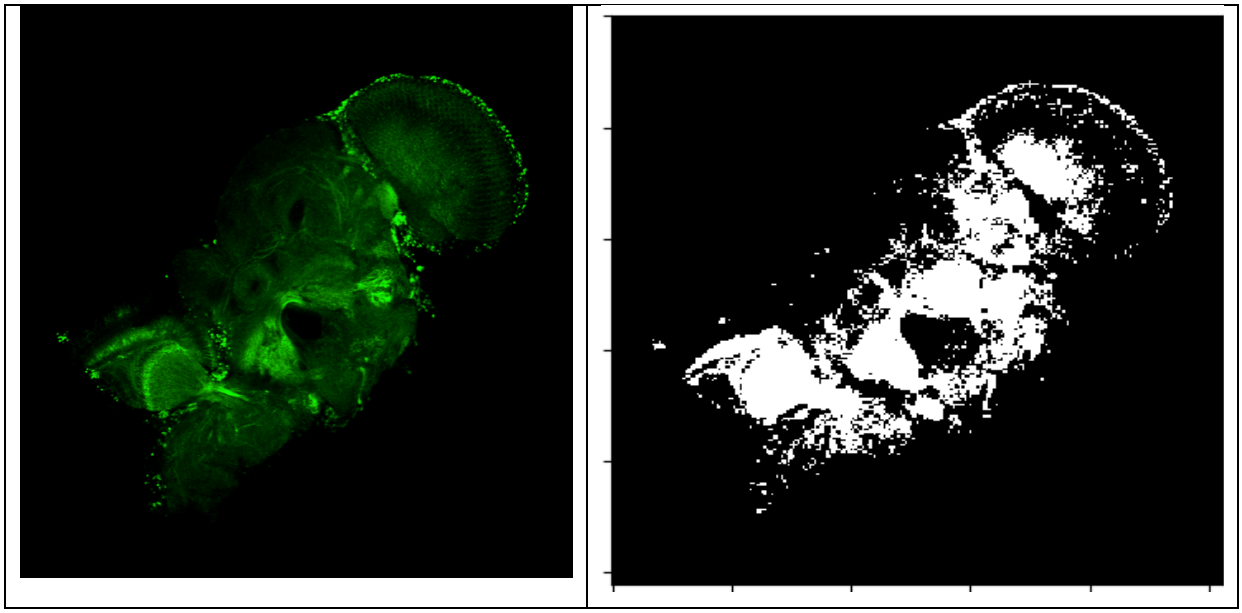


Figure 6 Bi-level segmentation of Fruit Fly image using modified Valley emphasis method Th=28

3.3 2D Histogram based methods-I

A 2D histogram is a two-dimensional matrix. Every element in the matrix represent the total number of times x(pixel value)and y (pixel value obtained when some operation is performed on the neighboring pixel values, for example variance, neighborhood averaging, weighted -mean and median) occur jointly in the image. Gray level gradient magnitude, gray level co-occurrence matrix and gray level spatial co-occurrence matrix are some more methods that use local information. In order to segment the given image using its histogram, we calculate different types of entropies like Shannon entropy, Renyi entropy, Tsallis Harvrds – Charvat entropy and Masi entropies for class one and class two using 2D histogram or 2D gray level co-occurrence matrix for threshold value computation

One of the drawbacks of 1-D histogram is that the spatial correlation between different gray levels is not considered. Hence, Abutaleb [53] extended the 1-D histogram by using the local average gray value of the pixels. Image thresholding using the 2D histogram suffers from the drawbacks of large time consumption and information loss during the optimal threshold search.Hence, Xiao et al [59] proposed gray level spatial correlation (GLSC) [58] and gray level gradient magnitude (GLGM) histograms that takes image correlation into account in a different way from the 2D histogram method.

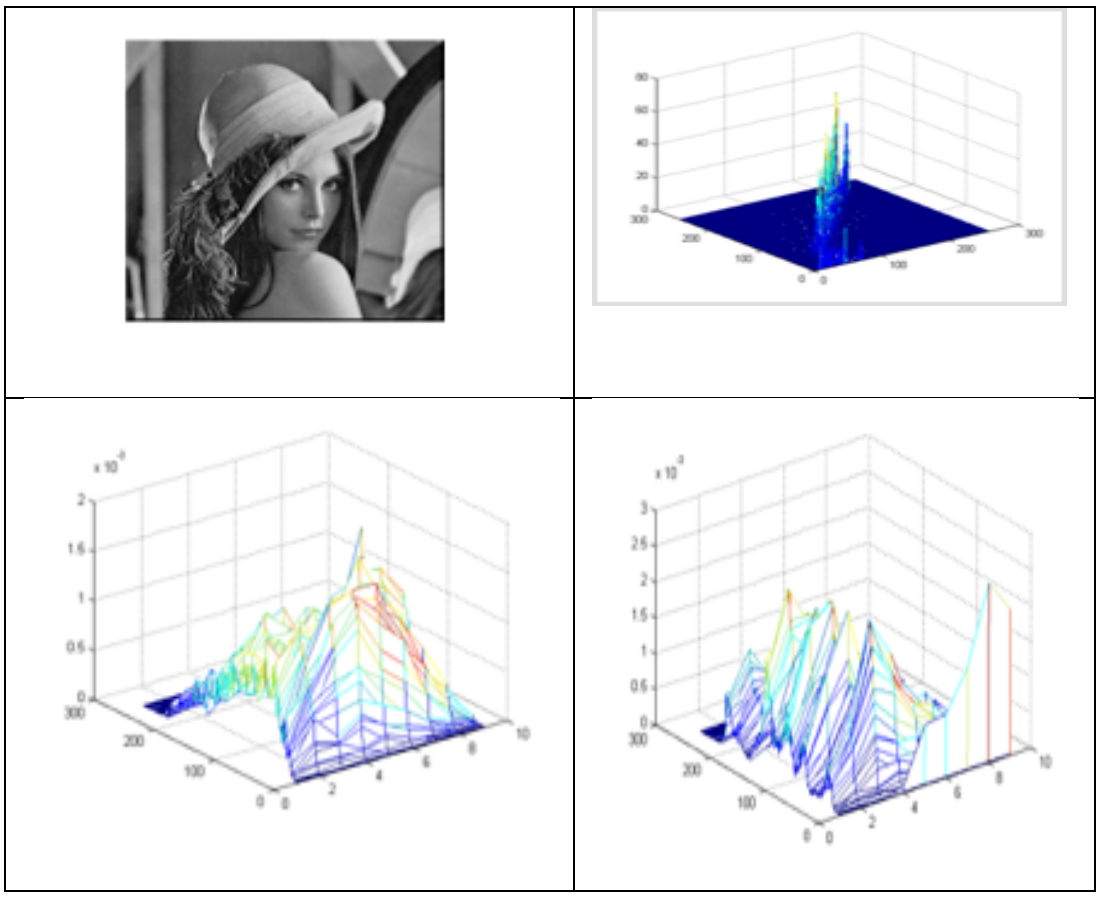


Figure 7 3D plot of 2D histogram of a) Lena image (TR) b) 2D histogram (TL) image vs mean filter c) GLSC histogram (BL) d) GLGM histogram (BR)

According to Xiao's research [58] image thresholding using these two histograms achieve equivalent or even better segmentation performance as compared with 2D histogram-based methods, while saving time remarkably, because the histogram size is less than the standard 2D histogram. These histograms are shown in Figure 7. Recently, Sanjay Agrawal et al [55] proposed a multilevel image thresholding technique using Coral Reef optimization and 2D histogram computed using a gray level co-occurrence matrix and entropy. The main advantage of this method is to consider the edge information. A similar approach was

proposed by FangyanNie [56] using cross- entropy. In our work we extend the Agrawal et.al to other types of entropies like Tsallis-Havrda – Charva't entropy [37], Masi entropy [62] and Renyi entropy. Simulation results show the Renyi entropy performs better that other entropy-based approach. It is observed from the Figure 7, that the size of the histograms is not same. Hence, image segmentation using GLSC and GLGM methods needs lesser computational time than the 2D histogram method. The main advantage of a 2D histogram is that the possibility of using the pixel neighborhood information in constructing the image histogram. In a 2D histogram x and y axis is used for representing the image and filtered image pixels.Hence, the threshold vector (s,t) (s and t corresponds to threshold values for image and filtered image) divides the histogram plane into four regions say R1, R2 (top row) , R3 and R4 regions (bottom row) , when an image is segmented into two classes, whereas ,if the image is segmented into three classes, two thresholds (s_1, t_1) , (s_2, t_2)divides the 2D histogram into nine regions.First, component of the threshold vector in bi-level image segmentation problem, is for pixel intensity and the second component is another threshold for pixel average of pixels. Regions R2 and R3 are related to edges and noise, whereas regions R1 and R4 contain object and background information. Hence, only these regions are considered for threshold value estimation. Regions R1, R2, R3 and R4 are denoted by [0,t]x[0,s], [t+1,255]x[0,s], [0,t]x[s+1,255] and [t+1,255]x[s+1,255] for an array of size 255x255.

A 2D threshold function is given by

$$f_{(T,S)}(x,y) = \begin{cases} b_0 & if\ f(x,y) \le T \check{} g(x,y) \le S \\ b_1 & if\ f(x,y) > T \hat{} g(x,y) \le S \end{cases}$$

Where $0 \le b_0$, T, $b_1 \le L-1\ and\ (T,S)$is a 2D Threshold vector..

3.4. 2D Histogram Based Thresholding Techniques-II

In these methods, threshold value required for bi-level or multilevel segmentation task is calculated using an error function which consists of n terms; proportional to the number of classes. Each term is calculated using entropy and 2D histogram or 2D Gray level co occurrence matrix. Even though, there are other matrices, we have selected 2D histogram and 2D gray level co-occurrence matrices, because their performance is better than other methods. One of the first methods based on entropy and 2D histogram was proposed by Pun [79]. It was corrected and improved by Kapur et al [80]. This concept was further generalized to Renyi's and TsallisHavrds- Charvat entropy[60] , cross entropy[56] and Masi entropies

[62]. A detailed study and comparative analysis of entropy techniques were presented in the review article by C.L.Change et al [81].Let a threshold vector (s,t) segments the 2D histogram into four regions, top row R_1, R_2 bottom row R_3, R_4 respectively, only main diagonal regions are considered because other regions are related to edges and noise. Error functions for both bi-level and three levels using various types of entropies using 2D gray level and co-occurrence matrix is given below

E(s,t) =$H_1^\alpha(s,t) + H_2^\alpha(s,t)$

$$H_1^\alpha(s,t) = -\sum_{m=0}^{s}\sum_{n=0}^{t} p(m,n)\log p(m,n)\,, H_2^\alpha(s,t) = -\sum_{m=s+1}^{L-1}\sum_{n=t+1}^{L-1} p(m,n)\log P(m,n)$$

Optimal threshold (s^*, t^*) can be found by maximizing the above equation over all possible threshold values.

$$(s^*, t^*) = \max_{0\le s,t\le 1} E(s,t)$$

Renyi's entropy for bi-level segmentation problem is

$$H_1^\alpha(s,t) = \frac{1}{1-\alpha}\ln\sum_{m=0}^{t}\sum_{n=0}^{s}\left(\frac{P(m,n)}{P_2(t,s)}\right)^\alpha$$

$$H_2^\alpha(s,t) = \frac{1}{1-\alpha}\ln\sum_{m=t+1}^{L-1}\sum_{n=s+1}^{L-1}\left(\frac{P(m,n)}{1-P_2(t,s)}\right)^\alpha$$

$$P(m,n) = \frac{number\ of\ pixels\ having\ gray\ level\ m\ and\ n}{NxM}$$

$$P_2(m,n) + P_4(m,n) = 1$$

$$P_2(m,n) = \frac{number\ of\ pixels\ having\ gray\ level\ m\ and\ n\ in\ block\ 2}{size\ of\ the\ block}$$

We use only main diagonal blocks (block 2 and block 4) because other blocks contain information about edges and nose , the optimal threshold pair (t^*, s^*) can be obtained by maximizing the E(s,t) function

$$(s^*, t^*) = \max_{0\le s,t\le L-1} H_1^\alpha(s,t) + H_2^\alpha(s,t)$$

Entropy using Tsallis Havrds Charvat for bi-level image segmentation is

$$H_1^\alpha(t,s) = \frac{1}{1-\alpha}\left[\sum_{m=0}^{t}\sum_{n=0}^{s}\left(\frac{P(m,n)}{P_2(t,s)}\right)^\alpha - 1\right]$$

$$H_2^\alpha(t,s) = \frac{1}{1-\alpha}\left[\sum_{m=t+1}^{L-1}\sum_{n=s+1}^{L-1}\left(\frac{P(m,n)}{P_2(t,s)}\right)^\alpha - 1\right]$$

Total Tsallis – Havrda – Charvat (THC) entropy of an image is

$$H_T^{\alpha} = \frac{1}{1-\alpha}\left[\sum_{m=0}^{L-1}\sum_{n=0}^{L-1} P(m,n)^{\alpha} - 1\right]$$

where $\alpha\,(\neq 1)$ is a positive real parameter. THC entropy is non-additive, hence, the criterion function for bi-level image is given by

$$E(s,t) = H_1^{\alpha}(t,s) + H_2^{\alpha}(s,t) + (1-\alpha)H_1^{\alpha}(s,t)H_2^{\alpha}(s,t)$$

Optimal threshold is computed by

$$(s^*,t^*) = \max_{0\le(s,t)\le(L-1)} E(s,t)$$

Thresholding based on Masi entropy

$$H_1^r(s,t) = \frac{1}{1-r}\log\left[1-(1-r)\sum_{m=0}^{s}\sum_{n=0}^{t}\frac{h(m,n)}{P_1(s,t)}\log\frac{h(m,n)}{P_2(s,t)}\right]$$

Where, h(m,n) = $\dfrac{number\ of\ values\ having\ gray\ m,n\ in\ a\ 2D\ histogram}{MxN}$

$$P_1(s,t) = \sum_{m=0}^{t}\sum_{n=0}^{s} h(m,n)\ P_2(s,t) = \sum_{m=t+1}^{L-1}\sum_{n=s+1}^{L-1} h(m,n)$$

$$H_2^r(s,t) = \frac{1}{1-r}\log\left[1-(1-r)\sum_{m=s+1}^{L-1}\sum_{n=t+1}^{L-1}\frac{h(m,n)}{P_1(s,t)}\log\frac{h(m,n)}{P_2(s,t)}\right]$$

Optimal threshold value can be determined by maximizing the below equation

$$(s^*,t^*) = \max_{0\le(s,t)\le(L-1)} H_2^{\alpha}(s,t) + H_1^{\alpha}(s,t)$$

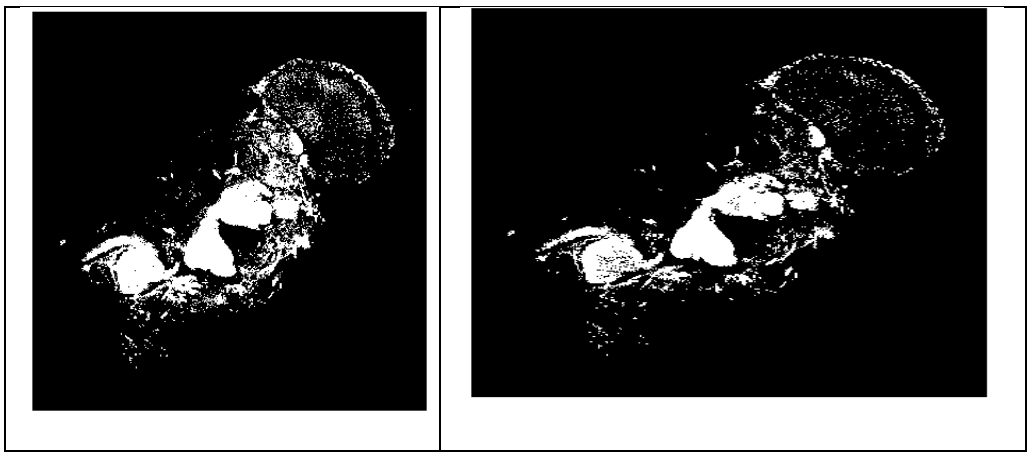


Figure 8 Entropy based Thresholding using a) Renyi method Th=34, alpha=0.7 b) Tsallis method Th=44, alpha=0.5

These methods are extended to multilevel thresholding problems [83]. Some of the extensions are minimum cross-entropy thresholding [55]. In order to, retain more edge information; gray level co-occurrence matrix was employed instead of 2D gray level histogram [56]. In general, one image is required for construction of Gray level co-occurrence matrix. This matrix was suggested by Haralick [82] for texture classification. It considers the relationship between two neighboring pixels. Let an image consists of L gray levels having a minimum value 0 and maximum value L-1. Gray level co-occurrence matrix P_d^θ for a displacement vector d=(dx,dy) and direction θ is defined as follows.

$$P_d^\theta(m,n) = \left[\left((r,s),(t,v)\right): f(r,s) = m, f(t,v) = n\right]$$

The elements at location (m,n) of P_d^θ is the number of occurrences of the pair of gray levels m and n which the distance between m and n following direction θ is d, Figure 9 displays an image its gray level co-occurrence matrix P_d^θ with distance d=1 and direction $\theta = 0$

0	1	0	2
0	2	1	1
3	1	0	0
0	0	2	3

a) 4x4 image

2	1	3	0
2	1	0	0
0	1	0	1
0	1	0	0

b) GLC matrix

Figure 9. Co-occurrence matrix calculation a) image b) gray level co-occurrence matrix for d=1 and theta=0 degrees

Figure 10..shows the log plot of gray level co-occurrence matrix of R, G and B images of a Mandrill color image. In this work like other authors work [55,56], we have constructed the gray level co-occurrence (GLC) matrix using average of four gray level co-occurrence matrices with distance d=1, and directions 0, 45, 90 and 135 degrees. All th entropy equations are same except (s,t) changes to (t,t) because we use one image for gray level co-occurrence matrix.

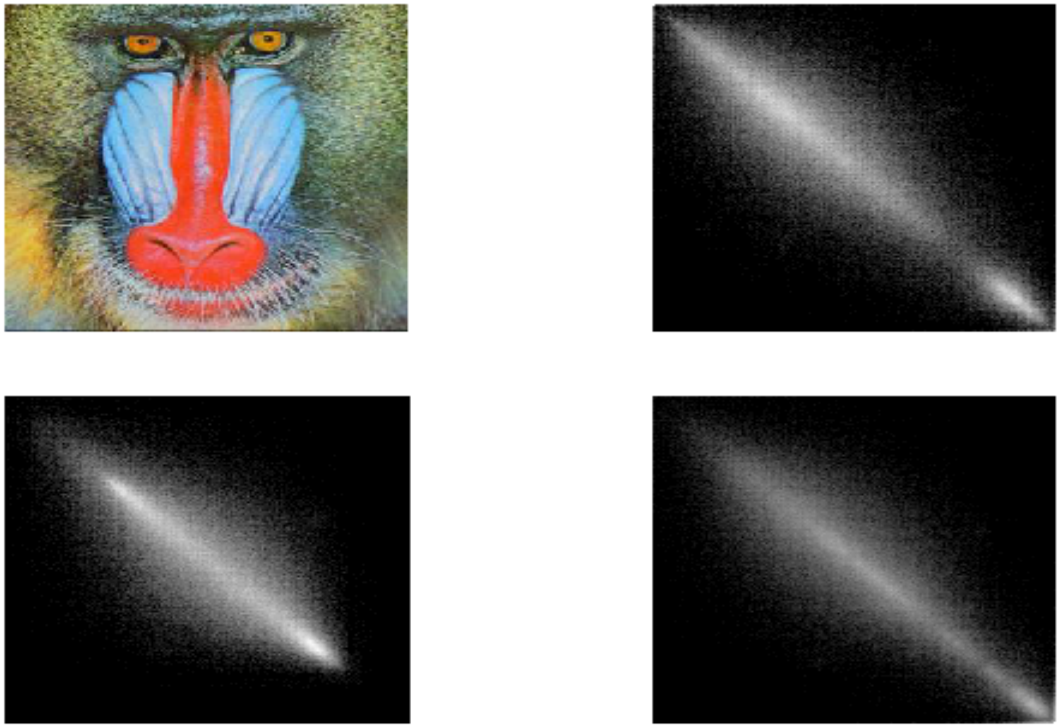


Figure 10 Gray level co-occurrence matrix of R,G and B images of a Mandrill image

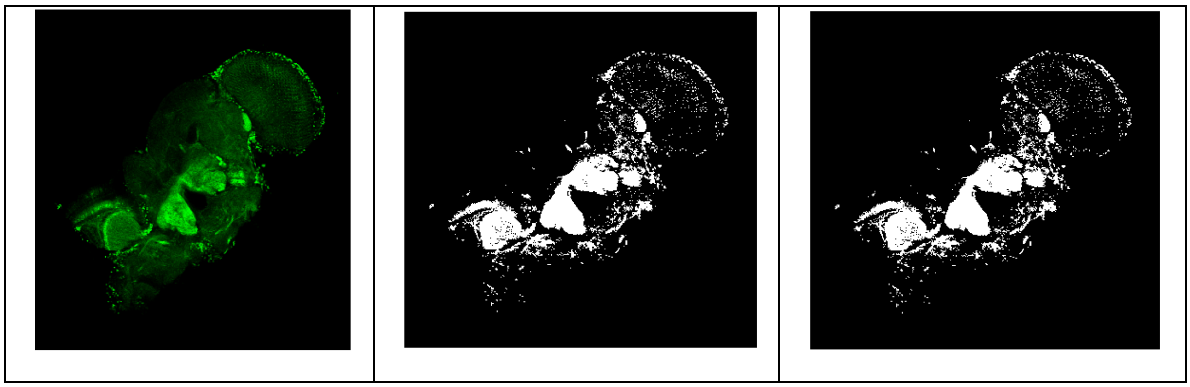


Figure 11 Entropy based thresholding method a) Original image b) Renyi entropy a1=0.6 Th=41 c) Masi entropy R=0.152 Th=41

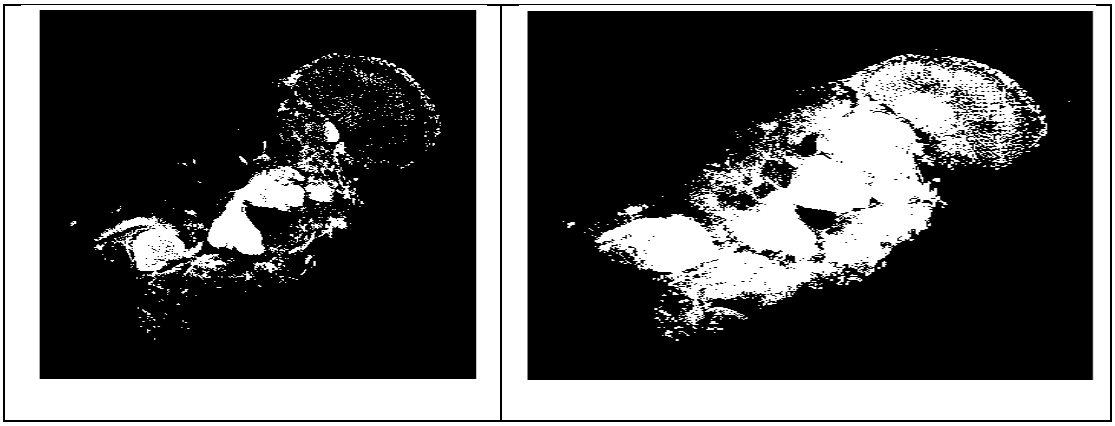


Figure 12 Entropy based thresholding a) Tsallis method q=0.6 b) entropy based method Th=16

3.5. Thresholding using Cross entropy: [84]

In this method an optimal threshold value is obtained by minimizing the information theoretic distance also known as directed divergence or cross entropy between object (R1) and background (R2) regions. Cross entropy between two distributions P and Q having L pixel values defined [56,84,] as

$$D(p,q)=\sum_{m=0}^{L-1} p(m)\log\frac{p(m)}{q(m)}$$

The above equation is asymmetric and its symmetric version is employed for image segmentation. Total symmetric cross entropy of an image segmented into object and background regions at threshold value't'. Let the observed probability distribution of object and background regions are P1 and P2. Let Q1 and Q2 be the probability distributions of the object and background regions based on some model. The total cross entropy of the of the image is

T(t)=D(P1,Q1)+D(P2,Q2) then the symmetric version on the total entropy is

$$T(t)=\sum_{x=1}^{t} P1(x)\log\frac{P1(x)}{Q1(x)}+\sum_{x=1}^{t} Q1(x)\log\frac{Q1(x)}{P1(x)}+\sum_{x=t+1}^{L} P2(x)\log\frac{P2(x)}{Q2(x)}+\sum_{x=t+1}^{L} Q2(x)\log\frac{Q2(x)}{P2(x)}$$

In order to segment an image we minimize the above expression w.r.to all values of t. In this expression we need to obtain Q1 and Q2 using some model. Nikhil R. Pal employed Poisson distribution [84] and Gamma distribution by AI_Ajlan [85].This method is computationally expensive.

3.6 Simulation Results:

In this chapter spatial thresholding methods are implemented using open CV python 3.2 software installed on MacBook Air, Laptop. Threshold values obtained are shown in the Figures 8,11, and 12 respectively.. Parameters are selected such that one obtains good results. Except the entropy based method all other methods provided good results. Threshold values obtained for different methods are indicated below the threshold image. It is observed that gray-level co-occurrence matrix method performed better than 2D histogram. In general Renyi entropy based method performed better than other methods. In figure 7 , TL, TR, BL and BR denotes top left, top right, bottom left and bottom right figures

CHAPTER 4

Contrast Enhancement of Confocal Images

The principal objective of image enhancement is to process a given image so that the result is more suitable than the original image for a specific application [12]. One normally, apply contrast enhancement technique on those images that are captured in a poor illuminating conditions or taken with a sensor having nonlinear gain or captured in a non-uniform illuminating condition. Some of the areas where these techniques are useful are in digital photography, medical image analysis, remote sensing and microscopy images etc. Several image enhancement techniques are proposed over the last decade and they are grouped into two categories: Spatial and Transform domain techniques. Some of the spatial domain techniques are histogram equalization [87], Gray level grouping [88,89]. Transform domain techniques modify the transform coefficients obtained either using Fourier transform or discrete cosine transform or wave form. Some of the well-known techniques are Alpha Rooting and contrast enhancement using DCT [86,90,91].

In recent years, both discrete and continuous orthogonal moments such as Geometric, Legendre, Zernike, Tchebichef, Hahn, Krawtchouk and Gaussian Hermite moments (GHM) are applied for solving image watermarking, Human Gait recognition, content base image retrieval, texture classification[92], Finger print classification[96] License plate character[93] recognition, stereo matching [94]and moving object recognition[95] etc. In this work, we use Discrete Gaussian moments for image contrast enhancement because they are defined using Gaussian function, this also helps to provide good results in the presence of noise. Hence, they are scale dependent. Further, the moment matrix size is less than the image size. Therefore image can be reconstructed with less number of moments. Finally, the base functions of DGHM are less sensitive to noise due to Gaussian function is used. Next we present Discrete Gaussian Hermite Moments and recurrence equations for generating them.

4.1 Discrete Gaussian Hermite Moments

These moments are constructed using both Hermite and Gaussian functions, there fore we define them in the next section

4.1.1. Hermite Moments

Discrete Gaussian Hermite Moments are constructed using Hermite polynomials and a Gaussian function. Hermite polynomial of degree p over the domain $(-\infty,\infty)$ is defined by

$$H_p(x) = (-1)^p \exp(\mathrm{x}^2)\frac{d^p}{dx^p}\exp(-x^2)$$

These polynomials are orthogonal with respect to a weight function $\exp(-\mathrm{x}^2)$ and their orthogonality condition is given by

$$\int_{-\infty}^{\infty} \mathrm{H}_p(x)\,\mathrm{H}_q(x)\exp(-x^2)\;dx = \begin{cases} 0 & p \neq q \\ 2^p\, p!\,\sqrt{\pi} & p = q \end{cases}$$

Recursive equation to calculate Hermite polynomials for different orders are given by

$$H_p(x) = 2x\mathrm{H}_{\text{p-1}}(x) - 2(p-1)\mathrm{H}_{p-2}(x)$$

where $\mathrm{P} \geq 2 \quad \mathrm{H}_0(x) = 1 \quad \text{and } \mathrm{H}_1(x) = 2x$

4.1.2 Gaussian Hermite Moments

Given a scale parameter σ Gaussian Hermite function of order p is defined as

$$\hat{\mathrm{H}}_{\mathrm{p}}(\frac{x}{\sigma}) = \mathrm{H}_{\mathrm{p}}(\frac{x}{\sigma})\exp(\frac{-x^2}{2\sigma^2})$$

In this expression σ represents the standard deviation of the Gaussian function. Based on these functions Gaussian Hermite Moment [97] for a continuous image f(x,y) of order p+q is given by

$$M_{pq} = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} f(x,y)\hat{H}_p(\frac{x}{\sigma})\hat{H}_q(\frac{y}{\sigma})dxdy$$

$$= \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} f(x,y)H_p(\frac{x}{\sigma})H_q(\frac{y}{\sigma})\exp(-\frac{x^2+y^2}{2\sigma^2})\,dx\,dy$$

For a digital image X (i , j), defined over a square region $[0 \leq i, j \leq$ N-1] , the expression to calculate DGHM of order (p+q) is

$$M_{pq} = \sum_{i=0}^{N-1}\sum_{j=0}^{N-1} \hat{H_p}(i)\hat{H_q}(j)X(i\,,j)$$

Given these moments of maximum order$(N_{\max}, N_{\max})$, digital image X (i, j) can be reconstructed using the equation

$$\overline{X}(i,j) = \sum_{p=0}^{(N_{\max}-1)}\sum_{q=0}^{(N_{\max}-1)} \hat{H}_p(i)\hat{H}_q(j)M_{pq}$$

$\hat{H_p}(i)$ and $\hat{H_q}(j)$are calculated using the expression given below.

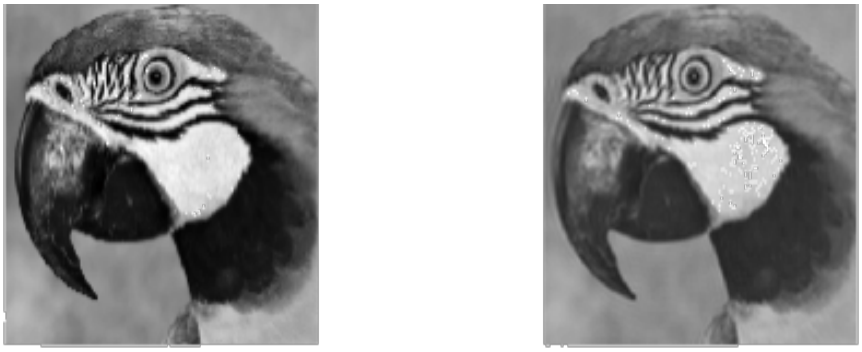
$$\hat{H}_p(i) = [(N-1)/2]^{-(1/2)}.\hat{H}_p(\frac{x}{\sigma})$$

$$\hat{H}_q(i) = [(N-1)/2]^{-(1/2)}.\hat{H}_q(\frac{y}{\sigma})$$

In order to apply these equations for moment computation, image coordinates are to be normalized $[-1 \leq x, y \leq -1]$ by

$$x = (2i - N + 1)/(N-1), y = (2j - N + 1)/(N-1)$$

Figure 13.show the Parrot image (256x256 size) reconstructed with Discrete Gaussian Hermite Moments of size 128x128.



a) Image1 b) Image2

Figure 13 Image Reconstruction a) Original Image b) Gaussian Hermite moments based method

4.2 Contrast Enhancement using DGHM

In this approach, local contrast defined using DGH moments is employed for contrast enhancement, for this purpose, we, find discrete Gaussian moment transform of the given image using Eq. 1, we use only 128x128 Gaussian Hermite moments, because image reconstruct is good for these moments, these are divided into 255 different frequency bands as shown in Figure 14 .Local contrast at n^{th} frequency band ($n \geq 1$) is defined as

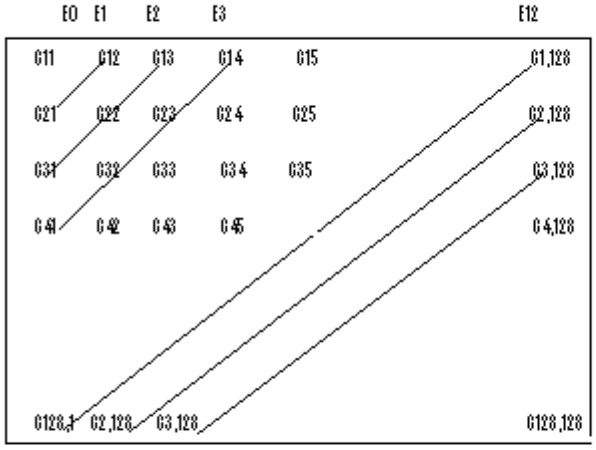


Figure 14 Diagram showing the different frequency bands

$C_n = \frac{E_n}{\sum_{t=0}^{n-1} E_t}$ Where $E_t = \frac{\sum_{p+q=t} |G_{p,q}|}{N}$ is the average of the coefficient magnitudes in each band.

N is the number of elements at each band and is given by

$$N = \begin{cases} t+1 & t < 128 \\ 254 - t + 1 & t \geq 128 \end{cases}$$

It can be shown [86] that the Enhanced Discrete Gaussian Hermite moment coefficients $\overline{G}_{p,q}$ *as*

$$\overline{G}_{p,q} = \lambda H_{p+q} G_{p,q} \qquad p+q \geq 1,$$

Where $H_n = \dfrac{\sum_{t=0}^{n-1} \overline{E}_t}{\sum_{t=0}^{n-1} E_t} \qquad n \geq 1$

$$\overline{E}_n = \lambda H_n E_n \qquad n \geq 1$$

$H_n (n = 1,2,....254)$ can be obtained by recursion. In this expression λ is an image enhancement control factor if $\lambda \succ 1$, the image will be enhanced and for $0 \prec \lambda \prec 1$ image will be softened. Enhance image is reconstructed using the modified moments.

4.3 Alpha- Rooting Technique

In this approach the magnitude of each DGHM coefficient is raised to a power α. Let G_{pq} be the DGHM coefficients of the original image and modified DGHM coefficient $\overline{G}_{p,q}$ is given by

$$\overline{G}_{p,q} = G_{p,q} \left| G_{p,q} \right|^{\alpha-1}$$

Figures 15 and 16 displays the enhanced images using DGHM , alpha rooting (α=0.98), Tchebichef and Krawtchouk moments based methods.

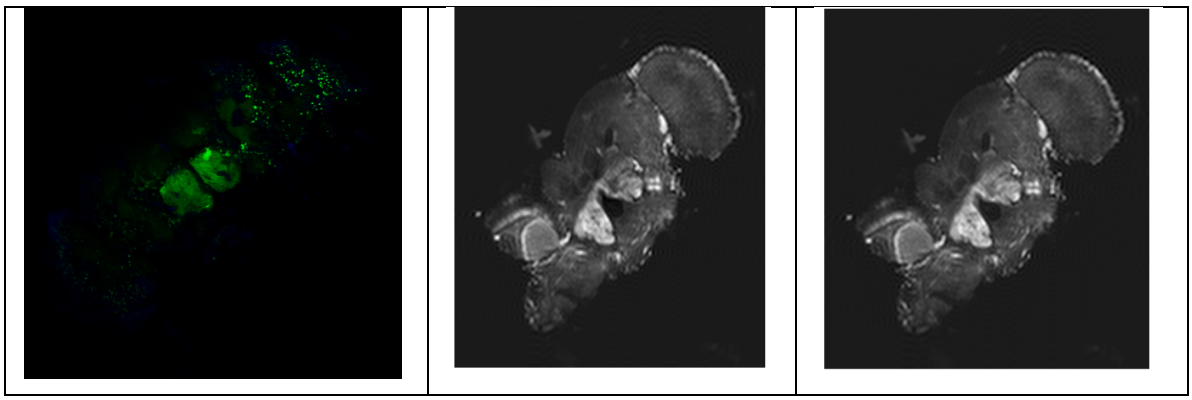


Figure 15. Enhanced Images a) Low contrast image b) DGHM method c) alpha root method

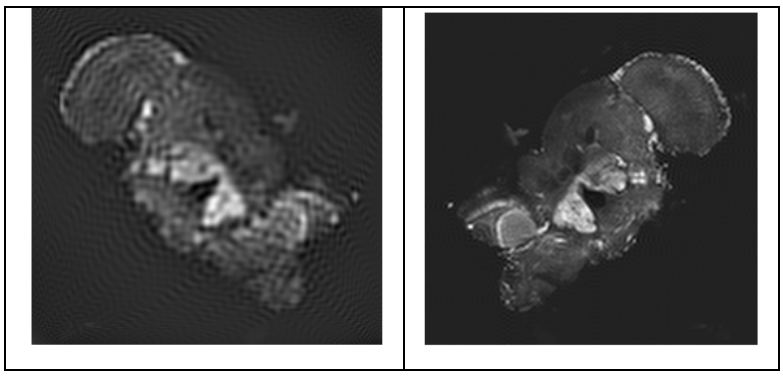


Figure 16. Enhanced Images a) Tchebichef moments b) Krawtchouk moments

METHOD	ENTROPHY	CO-INFO-CONTENT	UIQINDEX
ORIGINAL IMAGE	1.485	34.116	1
GAUSSIAN HERMITE MOMENTS	1.88	34.75	0.40
KRATCHOUK MOMENTS	1.82	27.0	0.315
TCHEBICHEF MOMENTS	2.23	10.68	0.170
ALPHA ROOTING WITH (GHM)	1.56	11.71	0.333

Table 1 Performance Evaluation of Enhancement Techniques

4.4 Simulation Results

Proposed approach was applied for monochrome fruit fly brain image, which did not come well due to low contrast, in order to improve the results, we employ contrast enhancement technique proposed in the previous section. Further, to verify the quality of the enhanced image we use entropy [98], Coefficient Information Content [98] and Universal Quality Index[99] was calculated for both original and enhanced images and the results are reported in Table 1.In this work we use σ=0.075.From the values reported in Table 1,one can conclude that the enhanced image using the DGHM is better than the other methods because entropy, coefficient of information content and universal quality index values are close to the original image. In this work, DGH moments are used for image contrast enhancement for confocal images and observed a slight change in the quality of the image when the sigma value is changed from 0.075 to 0.095 and 0.065. One can still improve the results by replacing the λ value by a function of the spatial frequency indices [100].
*

CHAPTER 5

De-noising and Edge detection of Confocal images

Moment based features have been employed for solving various problems in pattern recognition and image processing. In this chapter, we choose Charlier and Meixner moments separately, to solve image de-noising of confocal images corrupted with Gaussian noise. Proposed filter employ Non -Local Mean (NLM) filter with Charlier and Meixner moment's-based similarity values instead of pixel based similarity values that was used in NLM for filter weights calculation. Problems, either in image processing or in pattern recognition, require image representation by a set of values, invariant to various types of distortions. Even though, there are many methods available in the literature, orthogonal moment-based image representation gained popularity because of its good image reconstruction capability under different distortions and they are less sensitive to noise [101]. Hence, they are used as features for solving different problems in image processing and pattern recognition. Some of the well-known orthogonal moments used for image representation are Geometric, Legendre, Zernike, Tchebichef, Krawtchouk, Gaussian Hermite, Charlier, Meixner etc. Most of the reported work on Charlier and Meixner moments was related with image analysis and image reconstruction problems [102,103,104] only. Till now no attempt has been made to use them for denoising of confocal images corrupted with Gaussian noise. This motivated us to use these two moments for solving the above problem. In this chapter, we use these moments to solve de-noising of confocal images. using the Non-Local Mean (NLM) filter, that filters an image using local neighborhood patches. Earlier two filters were suggested using Zernike [105] and Krawtchouk [106] for cryo-EM images and SAR images. These two moments are also used for texture classification and image denoising problems by Perugu Ananth raj [123]In our approach, pixel weights are calculated using Charlier and Mexiner moments instead of pixel values used in the standard filter.

5.1 Discrete Orthogonal moments

In general, discrete orthogonal moments of a signal are obtained by projecting the sgnal onto a polynomial kernel function. In this work, we use both Charlier and Meixner kernel functions for defining these moments. Hence, a brief overview will be presented in this paper. These orthogonal polynomials are normalized to avoid fluctuations in the numerical calculations. Figure 17. Displays normalized Meixner and Charlier polynomials for N=256 points and order 10 obtained using a three-tern recurrence equation [101] .Recently, Cecilia Di Ruberto [107] et al used Stieltje's method to generate Legendre and Chebyshev polynomials and used them for texture analysis and image reconstruction problems. Modified Chebyshev algorithm [108] is another method used for generating orthogonal polynomials. This method is not that popular because selection of initial moments is a difficult task. Charlier polynomials are orthogonal with respect to the Poisson distribution on the non-negative integers [108] and Mexiner polynomials are also defined on the lattice N and orthogonal with respect to the negative binomial distribution [108]. One dimensional normalized Charlier $\breve{C}(p,a,x)$and Meixner $\breve{M}(p,\beta,\mu,x)$ polynomials of order p are defined [109,110] by

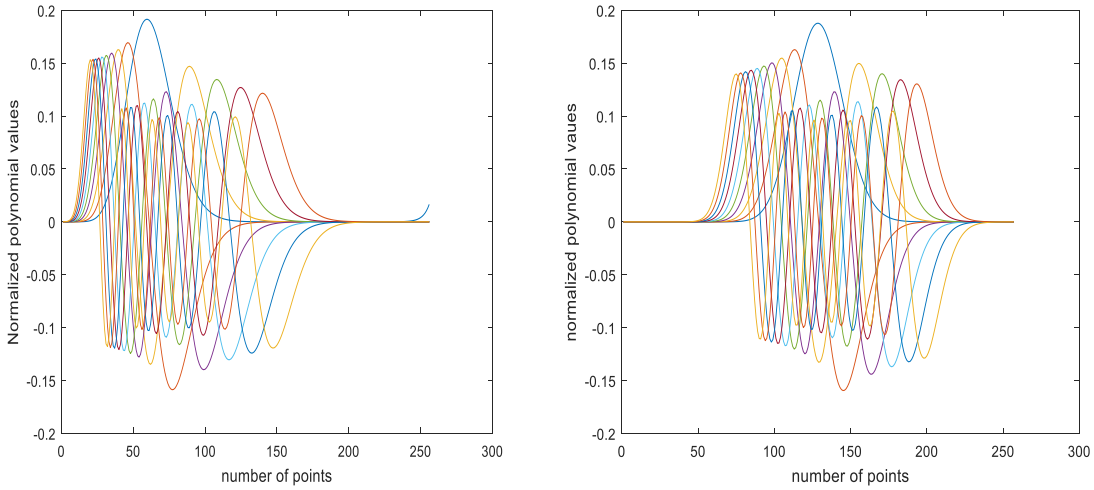


Figure 17. Plot of normalized Meixner and Charlier polynomials of order 10, N=256 points X axix in both figures denote no. of pints

$$\check{C}(p,a,x) = C(p,a,x)\sqrt{\frac{w(x)}{\rho(p)}}$$

Where $\rho(p) = \frac{p!}{a^p}$, $w(x) = \frac{e^{-a}a^p}{x!}$, a is a positive contant.

$$\breve{M}(p,\beta,\mu,x) = M(p,\beta,\mu,x)\sqrt{\frac{w(x)}{\rho(p)}}$$

$$\rho(p+1) = \frac{(p+1)(\beta+p)}{\mu}\rho(p)\rho(0) = \frac{1}{(1-\mu)^{\beta}}$$

$w(x+1) = \frac{\mu(\beta+x)}{(x+1)}w(x)$,w(0)=1

One of the important properties of these two polynomials are the availability of three term recurrence equation in terms of x and order n. First equation will be used for forward moment transform whereas the second equation will be used for inverse moment transform. Selected applications in this paper require only forward moments therefore we give an expressions for these moments of order (p+q) for an image F of size NxN only.

$$C_{pq} = \sum_{x=0}^{N-1}\sum_{y=0}^{N-1} F(x,y)\,\check{C}(x,p,a)\check{C}(y,q,a) \qquad (1)$$

Expressions for Meixner moments M_{pq}can be obtained by replacing $\check{C}(.)\ with\ \breve{M}(.)$ in the above expression. Three term recurrence, formulas are used to define these polynomials are normalized Charlier polynomials with respect to x

$$\check{C}(p,a,x) = \frac{\sqrt{w(x)}}{\sigma(x-1)+\tau(x-1)}\left[\frac{2\sigma(x-1)+\tau(x-1)-\lambda_n}{\sqrt{w(x-1)}}\check{C}(p,a,x-1) - \frac{\sigma(x-1)}{\sqrt{w(x-2)}}\check{C}(p,a,x-2)\right] \qquad (2)$$

Initial values required for solving the above equation are

$$\dot{C}(p,a,0) = \sqrt{\frac{w(0)}{\rho(n)}}C(p,\dot{a},1) = \frac{a-n}{a}\sqrt{\frac{w(1)}{w(0)}}\dot{C}(p,a,0),\ \sigma(x) = x, \tau(x) = a-x, \lambda_n = n$$

Normalized Maixner polynomials with respect to x

$$\breve{M}(p,\beta,\mu,x)=\frac{\sqrt{w(x)}}{\sigma(x-1)+\tau(x-1)}\left[\frac{2\sigma(x-1)+\tau(x-1)-\lambda_n}{\sqrt{w(x-1)}}\check{C}(p,\beta,\mu,x-1)-\frac{\sigma(x-1)}{\sqrt{w(x-2)}}\check{C}(p,\beta,\mu,x-2)\right]$$

(3)

Initial values required for solving the above equation are

$$\breve{M}(p,\beta,\mu,0)=(\beta)_p\sqrt{\frac{w(0)}{d_n^2}}.\ \breve{M}(p,\beta,\mu,1)=\frac{\mu(p+\beta)-p}{\mu\beta}\sqrt{\frac{w(1)}{w(0)}}\breve{M}(p,\beta,\mu,0)$$

$$(\beta)_0=1(\beta)_k=\beta(\beta+1)(\beta+2)\dots\dots.(\beta+k-1),k=1,2,3\dots.d_p^2=\frac{p!\,(\beta)_p}{\mu^p(1-\mu)^\beta}$$

$$\tau(x)=\beta\mu-x(1-\mu),\lambda_p=p(1-\mu),\sigma(x)=x$$

$$C_{pq}=\sum_{x=0}^{6}\sum_{y=0}^{6}F(x,y)\,\check{C}(x,p,a)\check{C}(y,q,a),\quad \text{p=0,1,2, q=0,1,2}$$

$$T_k(x,y)=\frac{1}{L^2}\sum_{(a,b)\epsilon W_{xy}}\left|tanh\left(\sigma(M_k(a,b)-\widetilde{M_k})\right)\right|,\text{k=1,2...6}\qquad(4)$$

.

Mathematical expression for a noisy image is

S(x,y)=F(x,y)+$\mathbf{N}(x,y)$

In this expression S(.,.) and F(.,.) represents noisy original images, whose size is NxN pixels, {x,y=0,1,…N-1},N(.,.) denotes the Gaussian Noise **,**with mean zero and variance σ^2. In this work, we use a Non-Local Mean filter [111] with feature-based similarity measure to process the noisy image. In the traditional Non-Local Mean filter, every noisy pixel is replaced by a weighted average (mean) of all the pixels within a pre-selected window, where the filter weights are computed using an neighbourhood similarity of the image patches. Filter weight between any two pixel position say x and y and processed pixel using NLM filter is given by

$$w(x,y)=exp\left(-\frac{[\![P_x-P_y]\!]^2}{h^2}\right)\qquad(5)$$

$$\tilde{S}(x,y)=\frac{\sum_{m=-a}^{a}\sum_{n=-b}^{b}w(m,n)F\,(x+m,y+n)}{\sum_{m=-a}^{a}\sum_{n=-b}^{b}w(m,n)}\qquad(6)$$

for x=0,1,…N-1 and y=0,1,2…N-1

Where P_x, P_y denotes image patchs at x and y respectively. Parameter h acts as a degree of filtering and it controls the decay of the exponential function. In our work these two values are replaced with Charlier,Meixner moment features. Sequence of steps required for filtering a noisy image using Charlier, Meixner moment features are

Step 1: Compute Charlier, Meixen moment at each pixel position of a given noisy image using 7X7 image patch

Step 2: Choose a similarity window of size 21x21 around each pixel position that is to be processed and compute the similarity weight between the pixel under consideration and all other pixel positions within a window of size 21x21 using Eq.5.

Step 3: Substitute the computed weight in Eq.6 to obtain the smoothed image.

In the next section, simulation results would be presents

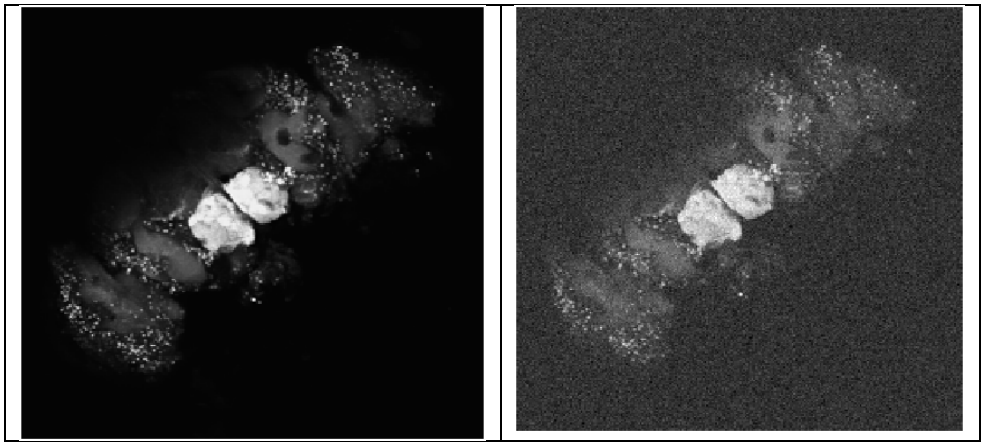


Figure 18 Images used in simulation a) Original image b) Noisy image

5.2 Zernike Moments based Edge detection method

Another important problem which belongs to segmentation category is the detection of edges in a given confocal images. In the literature, there are many edge detection approaches are available. In this report, we propose a orthogonal moment based complex operators to detect edges in the confocal images. This method, able to extract edge points both at sub-pixel and non-sub-pixels locations. Even though there are many moments available in the literature, we use Zernike moments of the image to detect edge points. The proposed approach use two masks one real and complex for pixel level edge detection and three masks, two real and one complex for sub-pixel edge detection. One dimensional edge model is shown in Figure 19.

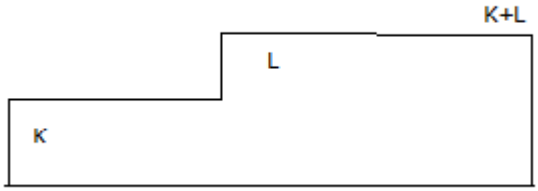


Figure 19 One dimensional edge model defined using K and h parameters

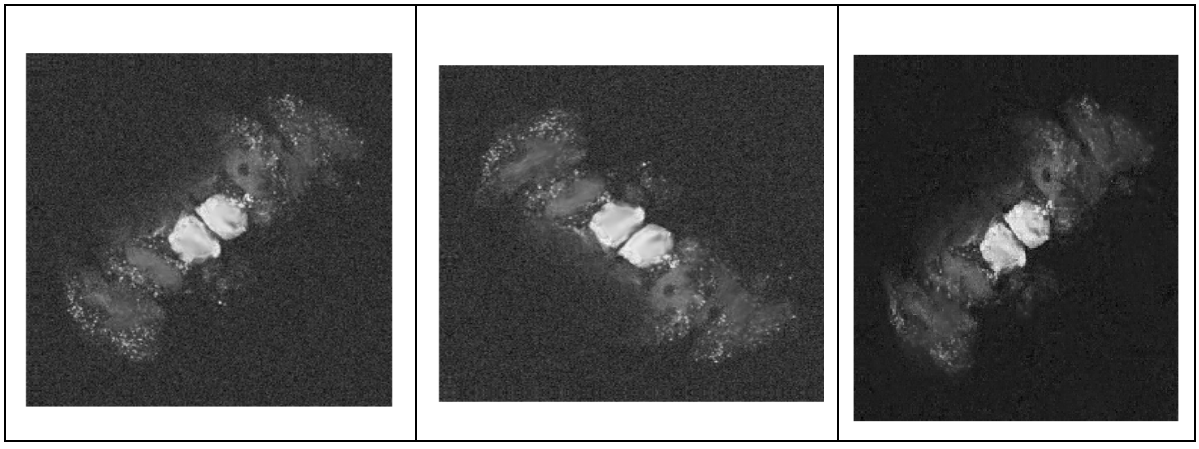


Figure 20 Filtered images using a) Charlier moments b) Meixner moments c) Gaussian Hermite moments

In order to find pixel edge points, the distance of the edge L from the centre of the mask is made to zero, then we require h and k parameters, which are given in terms two Zerike moments A_{00} and A'_{11}, these values are given in the reference [112].

$$k = \frac{3A'_{11}}{2} \text{ and } h = \frac{A_{00} - 0.5k\pi}{\pi}$$

Edge pixel is found if k is greater than a preselected threshold value. Results are shown in the Figure 21

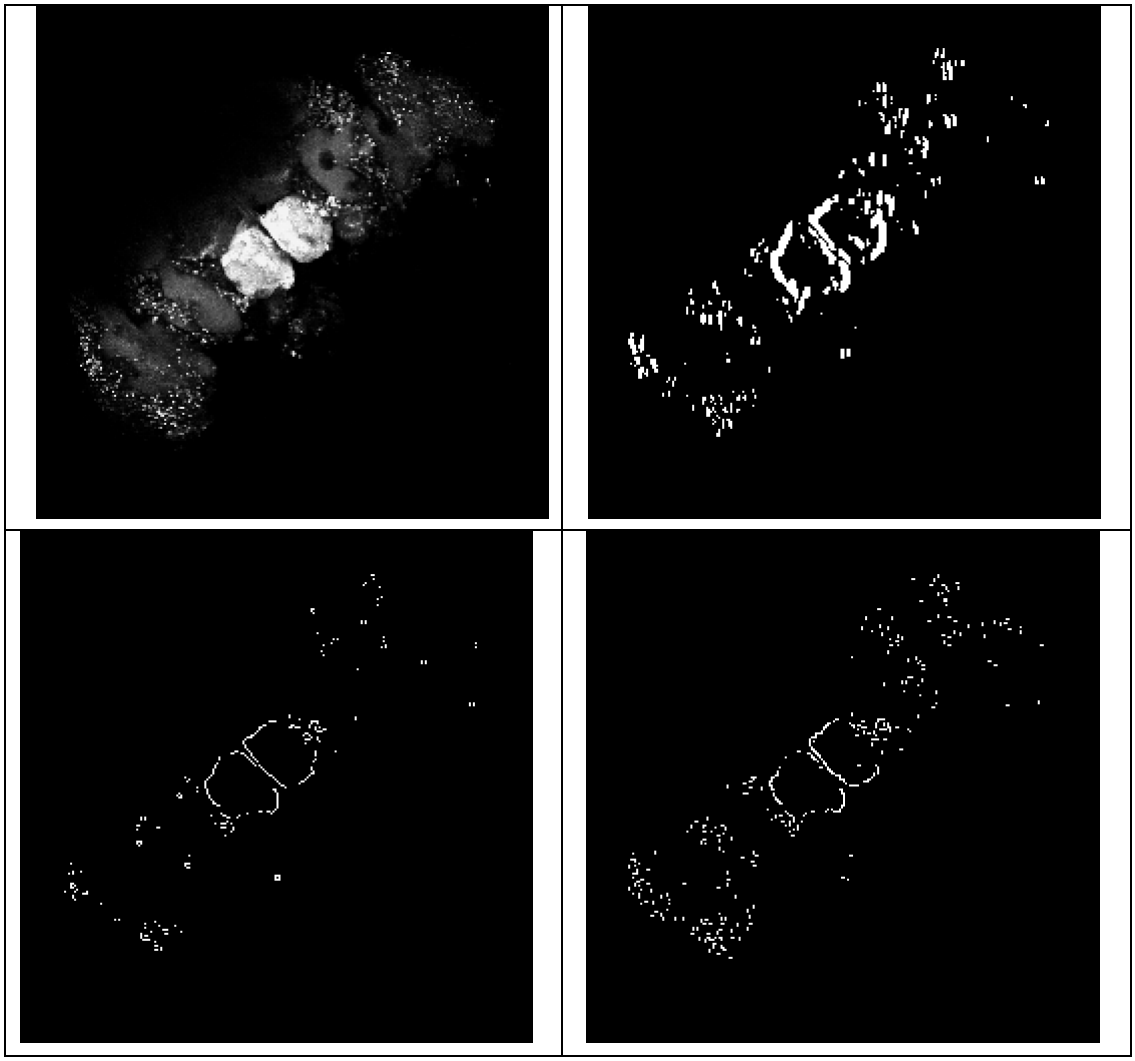


Figure 21. Edge detection a) Original Image (TL) b) Zernike moments (TR) c) Sobel (BL) d) Roberts operators (BR) Th = 40

5.3 Simulation Results

In order to test the proposed approaches for de-noising of images corrupted with Gaussian noise,. First given a confocal imag image was corrupted with Gaussian noise with sigma =0.02, both original and noisy images are shown in Figure 18.we have fixed a search window of size 21x21 pixels and a local window of size 7x7 pixels for computation of moments. We have used only eight moments for computation of filter weights and weight decay parameter h = 0.75*0.08 was used in our simulation. It is observed that when the h value is small “NO”

filtering is noticed, whereas for large value of h , we observed that blurring on the image. Hence, we have selected that value of h for which no blurring occurred. Filtered images are shown in Figure 20. In this paper we have used both Charlier and Meixner moment's based features for de-noising of images. Even though the filter worked well for uniform regions but its performance was not that good at edge locations. This can be solved using a regularization method. Finally, further work is required for processing images corrupted with other types of noise like impulse, Poisson and Speckle. Among the reported edge detector results shown in Figure 21, Zernike moment based method worked well, providing thick central portion of fruit fly brain image. We have used the same threshold value for all the edge operators.

CHAPTER 6

Objective Based Clustering Methods

The main task of data clustering, also known as cluster analysis is to split the given data into a number of non overlapping regions or clusters based on a measure of similarity such that the similarities between regions in the same groups are high while the similarities between different groups are low [34].Several clustering algorithms have been suggested in the literature with and without spatial constraints. These methods are grouped into fuzzy or non-fuzzy methods. In, non-fuzzy or 'Hard' clustering method, the boundary between the clusters is crisp, where as in fuzzy clustering approach; the boundary between the clusters is fuzzy. These boundary points are obtained using a fuzzy member ship function, which indicates different degree of belongingness. Fuzzy c- mean algorithm was introduced by Dunn [114] and later generalized by Bezedek.[115]. In this chapter, we use both c- means and fuzzy c-means techniques for clustering of fruit fly image into two regions. Further, we present some clustering methods that use spatial information.Even though we use the built-in functions available in MATLAB and OPEN CV software, but for understanding the procedures we explain them below

6.1. C-Means clustering method:

In this method, we find the cluster centers by minimizing the performance index (objective function), defined for c cluster centers $v_1, v_2, v_3 \ldots \ldots v_c$ and N data points X_k is given below.

$J_{CM} = \sum_{i=1}^{C} \sum_{k=1}^{N} u_{ik} \|X_k - v_i\|^2$ (1)

Intuitively, the above procedure is quite reasonable: first c clusters are guesses or taken as c centroids from the data set. These centroids are iteratively updated until the objective function is stabilized. Some more details can be obtained from the book [113].

The important component in the above sum is a partition matrix $U = [U_{ik}],\ i = 1,2,3,\ldots . c,\ k = 1,2\ldots . N$. whose role is to allocate the patterns to the clusters. The entries of U are binary. Pattern k belongs to cluster i when U_{ik}=1. The same pattern is excluded from the cluster when U_{ik}= 0. This matrix satisfies the following conditions [116]

1] Each cluster is non-trivia, that is it does not include all patterns and is non empty

$0 < \sum_{k=1}^{N} u_{ik} < N$ i=1,2,…..c

2] Each pattern belongs to a single cluster

$\sum_{i=1}^{c} u_{ik} = 1$ k=1,2,3…….N (2)

The objective function given in Eq. 1 with condition mentioned in Eq.2 is minimized with respect to $v_1, v_2 \ldots\ldots v_c \; and \; u_{11}, u_{12}, \ldots\ldots u_{cN}$ to determine the partition matrix elements and cluster centers.

$$v_i = \frac{\sum_{k=1}^{N} u_{ik} X_k}{\sum_{k=1}^{N} u_{ik}}$$

The main steps of c-means algorithm are as follows [113]

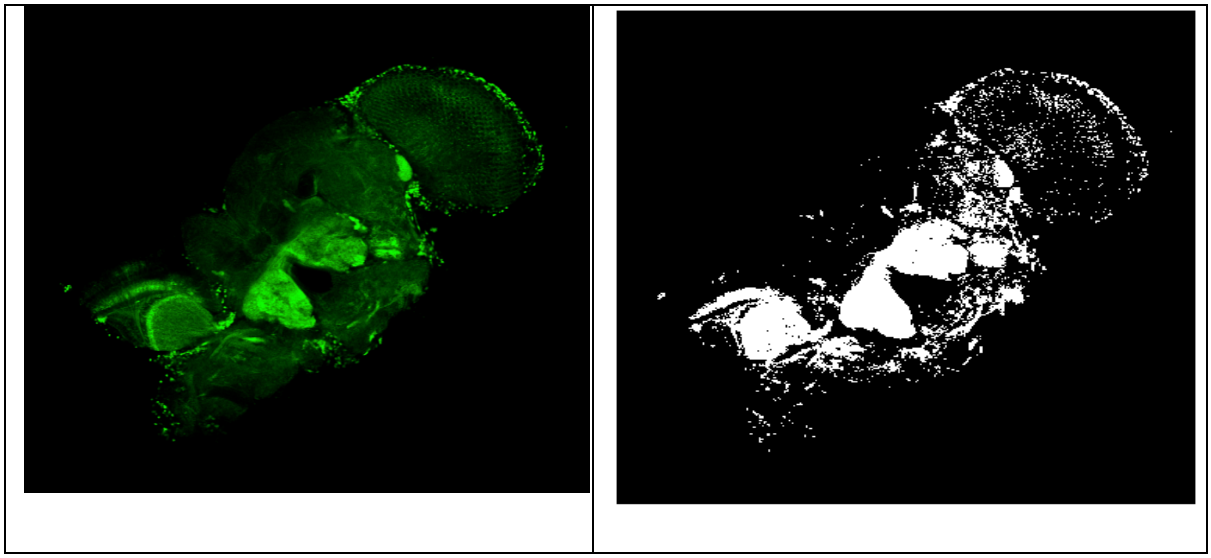


Figure 22. Image segmentation a) Original image b) c-means algorithm

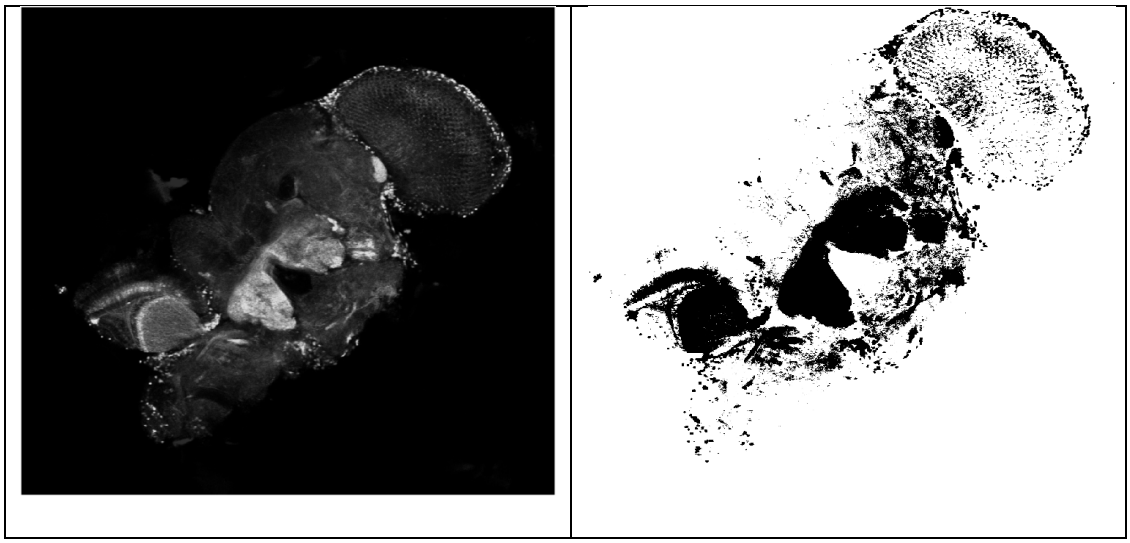


Figure 23. Image Segmentation a) Original image b) fuzzy c-means algorithm

Algorithm 1
begin initialize N, c, $\boldsymbol{v_1, v_2 \ldots\ldots v_c}$
do classify N samples according to nearest $\boldsymbol{v_i}$
re-compute v_i
until no change in v_i
return $\boldsymbol{v_1, v_2 \ldots\ldots v_c}$
end

6.2 Fuzzy c-Means clustering (FCM)

This clustering algorithm was proposed by Dunn [114] and further improved by Bezdek [115]. Mathematically, the standard FCM clustering algorithm partitions N data points X_k into c clusters by minimizing the objective function .The parameter m controls the fuzziness of the membership function. Cluster centers and partition matrix elements given by

$$J_{FCM} = \sum_{i=1}^{C}\sum_{k=1}^{N} u_{ik}^{m}\|X_k - u_i\|^2$$

$$v_i = \frac{\sum_{k=1}^{N}[u_{ik}]^m X_k}{\sum_{k=1}^{N}[u_{ik}]^m} \tag{3}$$

$$u_{ik} = \frac{(\|X_i - v_k\|)^{-\frac{2}{(m-1)}}}{\sum_{j=1}^{c}(\|X_i - v_j\|)^{-\frac{2}{(m-1)}}} \tag{4}$$

Main steps of the algorithm is given by

Algorithm 2 (Fuzzy k-Means clustering)

begin initialize N,c,m, $\boldsymbol{v_1, v_2}$ …. …. $\boldsymbol{v_c}$,$u_{ij} = 1$, i=1,2,……N, j=1,2,…c

normalize u_{ij}

do recomputed v_i using 3

recomputed u_{ij} using equation 4

Until small change in v_i, u_{ij}

return v_1, v_2 …. v_c

end

6.3 Fuzzy Clustering method with Spatial information

Fuzzy clustering method presented above does not using spatial information. Hence, Ahmed et. al [117] presented a different objective function that use spatial information is

$$J_s = \sum_{i=1}^{c}\sum_{k=1}^{N} u_{ik}^{m}\|X_k - v_i\|^2 + \frac{a}{N_R}\sum_{i=1}^{c}\sum_{k=1}^{N} u_{ik}^{m}\sum_{r\epsilon N_i}\|X_r - v_j\|^m$$

Computation of neighborhood information at each pixel require more computer time. Hence, Chen and Zhang [118] proposed a FCM_S1, a variant of the above method. The objective function is

$$J_s = \sum_{i=1}^{c}\sum_{k=1}^{N} u_{ik}^{m}\|X_k - v_i\|^2 + \alpha\sum_{i=1}^{c}\sum_{k=1}^{N} u_{ik}^{m}\sum_{r\epsilon N_i}\|\bar{X}_r - v_j\|^m$$

Where $\bar{X}_r$ denotes average of neighboring pixels within a window around X_r, , same authors replaced the neighborhood averaging with median. This method is not only reduces the execution time, but also improves the robustness to the Gaussian noise [118]. Another method, different from the Chen and Zhang[118] to speed up the first method was proposed by Szilagy et.al [119], this method is called Enhanced Fuzzy C-Means clustering. In this method, given image is changed to another image using

$$L_i = \frac{1}{1+a}\left(X_i + \frac{a}{N_R}\sum_{j\in N_i} X_j\right)$$

Where $L_i$represents the gray value of the i th pixel of th image L and N_i stands neighborhood at pixel i, a is constant parameter, N_R denotes the cardinality. New objective function using L image is

$$J = \sum_{i=1}^{N}\sum_{j=1}^{c} \gamma_i u_{ji}^m \left(L_i - v_j\right)^2$$

Where γ_i denotes the number of pixels having gray value i. The above function is minimized to find cluster centers and elements in the partition matrix. This algorithm provides comparable segmenting results to other methods, but the segmenting quality depends on the window size, the parameter a and the filter method. If the parameter a is large enough, then the method is resistant to noise otherwise not. Selection of correct values for a is difficult. Hence, Guoyint Liu et.al [120] proposed another Fuzzy c-means algorithm which includes local information adaptively. Recently, Soumyadip Dhar and Malay K.Kundu proposed multi-class image segmentation technique [121] using inter type-2 fuzzy sets

6.4 Simulation Results

In this chapter, both fuzzy c-means and c-means algorithms are employed for confocal image segmentation into two clusters. We have used a built in functions available in Matlab 2019 software. These programs are executed on Lenovo desktop having Intel I5 processor,. These functions for c-means and fuzzy c-means clustering are

[Unow, center, now_obj_fcn] = FCMforImage(img, clusterNum);

Result=kmeans(imagedata, no of clusters)

Results obtained are shown in Figures 22 and 23 respectively. From the results, we can say that Fuzzy c-means performed better than c-means algorithm.

CHAPTER-7

Conclusions and extensions

In this report, we have implemented image segmentation techniques for segmenting fruit fly brain images. Further, we have employed orthogonal moments such as Zernike , Gaussian Hermite, Charlier and Meixner moments for edge detection, noise filtering, contrast enhancement problems. Results show that these moments worked better than other moments for all the listed problems except noise filtering. It is due to not using a better similarity function. Even though, we have used it for Gaussian noise, it can easily be extended for other types of noise such as Poission and impulse noise. Clustering and Fuzzy c-means techniques worked better than thresholding techniques. These results can be improved using Fuzzy thresholding techniques and other region based methods such as Graph theory and active contour based approaches.

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